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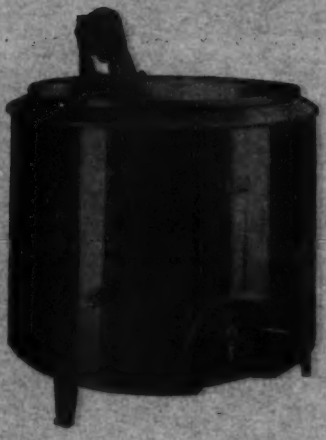

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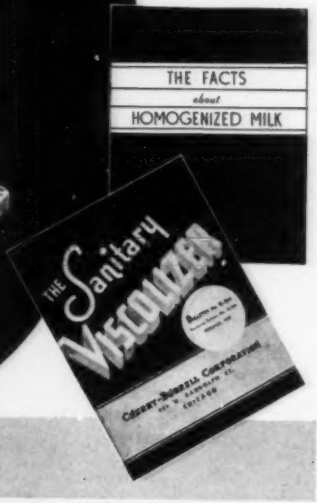

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JOURNAL OF DAIRY SCIENCE

VOLUME XXI MARCH, 1938 NUMBER 3

THE PREPARATION, PROPERTIES, AND USE OF GONAD-STIMULATING HORMONES[1]

L. E. CASIDA

Dept. of Genetics, University of Wisconsin, Madison, Wisconsin

The phenomenal results obtained from the use of gonad-stimulating hormones in the experimental laboratory have attracted widespread attention. It is a striking thing that a baby female rat can be made to ovulate 75 or 100 eggs when the best her mother ever did was to produce 10 or 12 eggs at one time. No other agent has been known which exerts such a clear and definite effect in speeding up the process of reproduction. People's minds have been challenged with the tremendous possibilities of modifying and controlling the process of reproduction in higher animals by means of these substances. Human medicine has been interested because of ever-present clinical problems which arise from aberrant sexual function. Animal husbandmen have seen in the discovery a possibility of dealing more effectively with the problems of sterility and lowered fertility in their livestock.

After the first report of the discovery, which was only about 10 years ago, work with the gonad-stimulating hormones has settled down chiefly to investigation of their sources, chemistry, and physiological action.

While the pituitary glands, body fluids, and certain other tissues of mammals, birds, and some lower animals all probably contain gonad-stimulating substances in some form and in some quantity, there are only a few sources from which sufficient quantity of the hormone can be obtained to justify their discussion here. The pituitary glands of hogs, sheep, cattle, and horses, which are slaughtered in large numbers at packing houses, constitute one important source. The urine of pregnant women and the blood serum of pregnant mares between the 40th and 80th days of gestation are also important sources. While there are other rich sources of hormones, such as foetal membranes and endometrium of pregnant mares, human placentae, urines of castrate men or women, menopausal women, and men or women with embryonal cell tumors, the sources themselves probably are not abundant enough to have widespread importance.

[1] Paper from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin. No. 215. Published with the approval of the Director of the Station. This work was supported in part by a grant from the Alumni Research Foundation.

Presented at the annual meeting of the American Dairy Science Association, June 21–25, 1937.

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The preparation of crude extracts from pituitary glands takes advantage of the solubilities of the hormones in water, dilute acids or dilute alkalis. After the active material is dissolved out of the tissue it can be removed from solution by precipitation with organic solvents such as alcohol or acetone, or by other substances such as tannic acid, tungstic acid, or ammonium sulfate. The active substance can also be adsorbed on such material as aluminum hydroxide. With repeated extractions and precipitations, meanwhile decreasing the volume of solvent, a fair degree of concentration and elimination of extraneous material can be brought about. Similar crude preparations from urine or blood serum rely first on precipitation and then repeated extractions and precipitations to bring about a partial purification. If certain precipitants are used, it may be necessary to employ special methods to free the active material from the precipitant, such as dialysis in the case of ammonium sulfate or treatment with brucine in the case of tungstic acid.

At least four different fractions of the pituitary extracts which have an effect on the gonads are at present recognized by some investigators. These have been characterized as follicle-stimulating, luteinizing, interstitial-cell-stimulating, and inhibiting in physiological action. The separation of the various fractions has been accomplished by fractional precipitation, usually by "salting-out," by increasing the hydrogen-ion concentration or by a combination of the two methods. No separation of extracts of urine from pregnant women into fractions showing different physiological action has been accomplished. Some evidence has been reported that pregnant mare serum extracts can be separated into follicle-stimulating and luteinizing fractions by methods similar to those used in fractionating the pituitary extract.

Physiological characterization of the various gonadotropic extracts has been made for the most part on the albino rat. In very general terms, the following reactions are obtainable in the ovary: (1) development of vesicular follicles to mature size or even larger, with accompanying female-hormone secretion; (2) ovulation of fertile eggs; (3) formation of functional corpora lutea; (4) luteinization of follicles without prior ovulation, thus forming corpora lutea atretica; and (5) development of interstitial tissue. The foregoing generalization must be qualified inasmuch as all of the responses mentioned are not invariably obtainable. For example, urine of pregnant women is relatively ineffective in hypophysectomized animals. Extracts from any source produce little effect in young animals before vesicular follicles form in the ovaries. Follicular stimulation can be brought about in younger animals than can ovulation or luteinization. Crude pituitary extracts tend to form atretic corpora lutea rather than corpora of ovulation. Graded dosages of pregnant mare serum have yielded quite different results: smallest dosages—follicular-stimulation; larger dosages—ovulation of a number of follicles normal for the species; still larger—superovulation; largest—formation of cystic follicles and atretic corpora lutea.

Mammalian testes have not responded to gonad-stimulating extracts to the same degree as mammalian ovaries. The most commonly recognized testicular response is that of increase in interstitial tissue and the accompanying hypersecretion of male hormone. The secretion of the testicular hormone has been assumed, more from the response of the accessory glands, such as the seminal vesicles, than from a marked increase of sexual desire. Consistent effects on the tubular tissue of the testes have not been reported. Some investigators have observed an increase in size of the seminiferous tubules and an increase in number of spermatocytes. Others, on the contrary, have observed actual tubular damage.

The situation with regard to the differential responsiveness of ovary and testis as observed in mammals appears to be reversed in birds. There the testes are quite sensitive to stimulation, even to complete spermatogenesis; whereas the ovaries are quite refractory. In some seasonal breeding mammals, however, such as the ground squirrel, the testes can be stimulated quite completely by gonad-stimulating extracts.

The various fractions of gonadotropic extracts are characterized, physiologically, by a preponderant action with relative, but not necessarily complete, absence of other actions. For example, a follicle-stimulating extract causes the maturation of follicles without formation of corpora lutea in only 2/3 to 3/4 of normal 21-day-old female rats. It has been postulated that the animals' own pituitaries have furnished the luteinizing action in the animals which form corpora lutea. Some evidence in support of this comes from the greater consistency of follicle-stimulation in hypophysectomized rats. The follicle-stimulating fraction when administered to males is said to be gametogenic, causing growth of the testicular tubules and development of their epithelium. The action appears to be more pronounced in restoring function in hypophysectomized mature rats than in hastening function in immature normal animals.

The action of the luteinizing fraction in the female becomes effective either immediately following the action of a follicle-stimulating preparation or in synergism with its action. Presumably, small vesicular follicles are not acted upon by the luteinizer. It is only as a follicular-stimulator develops the follicle nearly to mature size that the luteinizer, alone, or in interaction with the follicle-stimulating fraction, brings about the pre-ovulatory swelling of the follicle, ovulation, and corpus luteum formation.

Some investigators have considered the development of interstitial tissue in the testicle and the resulting secretion of male hormone as an action of the luteinizing fraction. Still other investigators believe this response can be attributed to a specific fraction, the interstitial-cell-stimulator, which produces relatively little luteinizing or follicle-stimulating action. In the female this fraction is effective in preventing the regression of the interstitial tissue of the ovary in hypophysectomized rats.

Another fraction of a pituitary extract is known as the gonadotropic antagonist. It can hardly be called gonad-stimulating in action inasmuch as

it inhibits the action of the follicular-stimulator. To be most effective it must be administered intraperitoneally rather than subcutaneously. It appears to act by causing atresia of follicles that might otherwise have been developed by the follicle-stimulating preparation, but has no inhibitory action upon luteinization.

The relatively specific actions of different gonad-affecting fractions have led to the postulation that the anterior pituitary gland secretes a number of different hormones affecting the gonads. Some of the fractions are spoken of as "hormones," without qualification. Hypotheses are made regarding the regulatory mechanism of the oestrous cycle. These are based on the action of certain pituitary hormones at certain times in the cycle, followed by the action of other hormones to take care of the next end-reaction observed in the ovaries. It seems only reasonable to point out in this connection that much of the experimental work which forms the foundation for these hypotheses has involved fractions of extracts and not "pure" hormones. Such preparations probably contain one or more hormones in unknown concentrations or proportions and in addition, undetermined amounts of substances which by themselves are relatively inert physiologically. The physiological action of the fraction as a whole, however, may be a result of a marked interaction of these inert substances with the primary substances in the preparation. In other words, a certain response from a pharmacological preparation does not necessarily imply a correspondingly identical substance acting in the physiological mechanism of a normal individual.

Two other types of substances, which are probably extra-pituitary in origin, should be considered at this time because they add complexity to the response of an experimental animal to hormone administration. The first of these may be classed as augmenters, inasmuch as they appear to increase the gonad-stimulating action when administered to immature rats of either sex in conjunction with some pituitary extracts. Augmenters include a variety of chemical substances such as hemin, casein, zinc, and copper salts, extracts of yeast, liver, muscle, lemons, etc. No entirely satisfactory explanation has been offered for the action of all these substances. Perhaps one of the most prevalent is that they prolong and slow up the rate of absorption from the site of injection or slow up the rate of removal of the hormone from the blood stream. The action of the augmenters in species other than the rat has not been adequately investigated.

The other type of modifying substance is the inhibitor. The antagonist of pituitary origin has already been mentioned. In addition to this, however, certain species have been found to build up anti-hormones in their blood serum when they are submitted to long-continued injections of the gonad-stimulating extracts. The anti-hormone exhibits itself by failure of the animal to respond further to the action of the hormone. It can also be demonstrated by the inhibiting or neutralizing effect when serum from a treated animal is mixed with the gonadotropic extract which induced the

anti-hormone and injected into test rats. The question arises as to the specificity of the anti-hormone. Some results seem to indicate that anti-hormones can be developed which will inhibit gonadotropic extracts from whatever source. Other results indicate that they are largely specific. Differences in the method of building them up and in the sensitivity of the test for them may account for the discrepancies.

While augmenters and inhibitors in the form in which they are being studied may not play any great part in the normal physiology of an animal, it is possible that they may have value for clinical use. One might conceivably use augmenters with the gonad-stimulating action of the pituitary gland in cases of hypo-gonadism and inhibitors in cases of hyper-gonadism.

The interest of this audience is doubtless centered on the possible use that gonad-stimulating hormones may have in correcting breeding troubles in dairy cattle. Those breeding difficulties in which infection is not the primary cause and in which abnormal gonadal function impairs fertility would appear to offer some possibility of responding to treatment with gonad stimulating hormones. Superficially, animals of this type might be placed in five classes: (1) cows that do not come into heat; (2) cows that come into heat more or less regularly but do not settle upon service; (3) nymphomaniac cows; (4) bulls that serve cows more or less readily but do not settle them; (5) bulls that will not serve a cow or are very slow in doing so.

A certain amount of breeding difficulty is encountered in any breeding herd. Attempts have been made to lessen these difficulties by injecting a part or all of the animals with gonad-stimulating extracts. In some of these cases the number or seriousness of breeding difficulties has actually increased. In other attempts only those animals which were showing some indication of breeding difficulty were injected. The results have been inconclusive. Some cows conceived; other appeared to be made worse; still others were unaffected as far as could be told. It has been found that gonad-stimulating extracts are effective in cows in bringing about practically all of the ovarian changes which would appear to be necessary, such as maturation of follicles, ovulation, formation of corpora lutea. The problem is to control these reactions so as to overcome the particular breeding difficulty involved.

Experimental work with rats has indicated that the qualitative and quantitative responses may be quite different with different dosages of gonad-stimulating extracts and different methods of administration. It is therefore necessary when applying these preparations to cows, that different dosages and different methods of treatment be studied. The cow presents a peculiar problem from the standpoint of hormone therapy because the ovulation of more than one egg at a time is highly objectionable. Gonad-stimulating extracts tend to develop a number of follicles, the degree of stimulation being related to the size of the dosage and the interval of treatment. It has been extremely difficult to bring a single follicle to maturity without other follicles being brought to a nearly comparable stage.

The cow herself is a second factor which renders control of hormone therapy difficult. It is not at all unlikely that every kind and degree of breeding difficulty will require a different treatment. Cows that may appear to have the same difficulty from the standpoint of behavior may represent widely diverse physiological conditions. As an illustration, it has been found at the Wisconsin Experiment Station that cows which fail to come into heat within a reasonable time after calving may have any one of the following pathologic ovarian conditions: (1) persistent corpus luteum; (2) quiescent ovaries, with neither follicles nor corpora lutea of appreciable size; (3) periodic development of anovulatory follicles, without heat; (4) periodic ovulation without heat. Even this type of analysis, with a knowledge of the ovarian response to hormones in the different conditions, may not be sufficient to give the control necessary for overcoming breeding difficulties. Each individual animal will doubtless need clinical study over a period of time to determine the indicated treatment and then the course of the treatment may have to be gaged to the response of the individual animal.

Investigation of this general problem at the University of Wisconsin during the past three years has been centered largely on the gonadal responses to hormonal treatment rather than on correcting breeding difficulties of herds in production. It was recognized that the animal's own endocrine system, and in particular the anterior pituitary, may complicate the study because of the additive or interactive effects of its own hormones. For that reason a part of the studies have been carried on with young calves, in which gonadotropic activity is probably at lower ebb than in mature individuals. It should be pointed out, however, that gonadal activity is exceedingly variable in calves even long before puberty. Very few calves of any age have shown a complete absence of macroscopically visible vesicular follicles and these have given no ovarian response to gonad-stimulating extracts. Ovulation has been brought about in other calves in three different ways: (1) injection subcutaneously of unfractionated pituitary extract, daily, for 11 or 12 days; (2) daily injection, subcutaneously, of pregnant mare serum for 6 days; (3) injection of follicular-stimulating extract, subcutaneously, on each of 2 to 6 days followed by administration of luteinizing extract intravenously on the succeeding day. The number of eggs ovulated has varied from 1 to 5 and many follicles which did not ovulate were definitely stimulated in each case.

Large quantities of sheep pituitary extract and of pregnant mare serum have been injected into bull calves one to four months of age over periods as long as 7 weeks but in no case have mature sperm been demonstrated. The size of the testicles was increased but it has not been definitely determined whether this was due to increase in interstitial tissue or size of seminiferous tubules or both. The seminal vesicles of these calves were definitely stimulated, which points to increased activity of the interstitial tissue. In

neither the bull nor the heifer calves has there been definite evidence of development of sexual desire even with extreme development of the gonads.

It is much more difficult to evaluate the effects of a gonad-stimulating extract when injected into mature cows. If the reproductive organs of the animals are normal, their ovaries and pituitary glands are changing periodically. If the animals are abnormal, it is very difficult to know which part of the endocrine system is primarily responsible for the condition. It may be possible that two animals which exhibit similar external behavior, and even similar internal behavior of the genital organs, are suffering from different fundamental defects of the endocrine system and will give different responses to the same endocrine treatment.

A general statement can be made that cows in all the different reproductive conditions which have been tried have responded in some degree to gonadotropic extracts. These include cows in the follicular and in the luteal phases of the oestrous cycle, in different stages of pregnancy, in the immediate post-partum period, cows with cystic ovaries, quiescent ovaries, and ovaries containing retained corpora lutea. The quantitative response to a standard dosage probably varies widely in animals representing the various foregoing pathologic conditions.

An entirely satisfactory measure for a dosage of gonadotropic extract is lacking. As long as the hormone is not in a chemical form that can be measured physically, there appear to be only two possible ways of expressing quantity of hormone. One is in equivalence of tissue or body fluid from which the hormone came and the other is in units determined by degree of response in small laboratory animals. Both methods are subject to criticism but the latter method is generally preferred because it gives some expression of the potency of a preparation. It is objectionable, however, in that it measures activity in a species other than the one in which it is to be used. Equal rat unit dosages of unfractionated pituitary extract and of pregnant mare serum give quite different results in cattle, fewer rat units of pituitary extract being needed to produce a response. Even among pituitary extracts there is some indication that similarly prepared extracts, administered in equal dosage in terms of rat units, may give quite different quantitative responses in cattle. This general situation may be due in part to error of the biological assay in each of the species; it may also be that undetermined interacting substances may affect the response in one species and not in the other.

Superovulation has been brought about in cows by subcutaneous injections of unfractionated pituitary extract just before an expected heat period. As many as 19 follicles have been ovulated from such a treatment. Although the ovaries have been enlarged by the treatment in all cases, in some only one follicle has been ovulated and in others luteinization of follicles has occurred without any ovulation. The conclusion is that the control of ovulation by this treatment does not appear satisfactory.

It should be noted at this point that workers at the California Experiment Station report ovulation being produced in the cow within 72 hours after the administration of pregnant mare serum.

Probably the surest method of producing ovulation with unfractionated pituitary extracts is by subcutaneous injections for one to seven days to grow the follicles and then intravenous injection of the same preparation two days later to produce ovulation. Ovulation has occurred within 36 to 48 hrs. after the intravenous injection. To a certain extent the number of follicles matured and subsequently, the number of eggs ovulated appears to be controllable by the total dosage given subcutaneously. But even by reducing the dosage the ovulation of a single egg is not insured. A single intravenous injection of the pituitary extract has not produced ovulation even though there has been a mature-sized follicle in the ovary, developed by the animal's own hormonal action.

It is doubtful if a single heat period has been observed in the experimental animals which can be ascribed to the hormonal treatment. A complete set of ovarian changes can be brought about and yet the cow shows no inclination to accept the bull. In some cases when the treatment has been near the time of the cow's expected heat period, she has accepted the male satisfactorily. Such heat periods are sometimes extended in length or they may be intermittent for a few days. The situation seems to emphasize the need for study of the physiological factors which condition the oestrous behavior of the animal as well as of those that bring about the ovarian changes.

All these results make it clear that there is much we do not understand about the action and standardization of gonad-stimulating hormones, let alone their effective use and control. Therapeutic use of these substances will undoubtedly sometime have a place in dealing with cases of sterility and lowered fertility in dairy cattle. At the present time, however, we are to a large extent dealing with preparations of unknown purity and unknown strength. Quite variable results have been obtained from administration of these preparations to different cows. Preparations of consistent strength and purity are highly desirable. It also seems necessary to know more about the altered physiology of the reproductively abnormal animal and the responses of each specific kind of abnormality to different methods of treatment (medical or surgical). Although successes from use of these materials on such animals are largely a result of chance at the present time, systematic study of these cases so as to recognize clinical entities should eventually give greater control in obtaining the desired results. Another outcome which should result from investigation of this field is the discovery of causes of reproductive abnormalities and subsequently, means to prevent their development. Such an outcome is even more important than the perfection of hormonal therapy for breeding difficulties.

A COMPARISON OF FRESH AND FROZEN CONDENSED SKIMMILK AS A SOURCE OF SERUM SOLIDS IN ICE CREAM[1]

E. L. REICHART AND R. T. CORLEY[2]

INTRODUCTION

In ice cream making it has been customary to use concentrated milk products such as plain condensed skimmilk, sweetened condensed skimmilk, and skimmilk powder in order to build up the serum solids content of the mix. This has been done in order to improve the texture and body of the ice cream, and because such concentrated milks are a relatively cheap source of solids.

As there are certain seasons of the year when milk supplies are plentiful while at others there is a shortage, it would be desirable to store milk solids with a minimum amount of processing prior to storage.

Milk solids-not-fat can be stored in various forms; the possibility of storing serum solids in the form of frozen condensed skimmilk has been tried and found quite successful, as a commercial practice, in the University Creamery.

STATEMENT OF PROBLEM

The purpose of this study was to study the effect of using frozen condensed skimmilk of good quality in ice cream.

Since the length of time taken to reach a certain percentage or a maximum overrun during the freezing process, and the quality of the resulting ice cream are the two most important points in which the average manufacturer is interested, any differences in these points obtained as a result of the use of frozen condensed skimmilk in ice cream mixes, were especially noted. Any other observable variations in the ice cream mixes themselves were also carefully noted. Therefore this study was a tabulation of the changes occurring in ice cream mixes in pH, titratable acidity, protein stability, whipping ability, and quality from the standpoint of flavor, body and texture, and resistance to melting, that took place as a result of the use of frozen stored condensed skimmilk as a source of serum solids. No attempt was made to study the problem from a bacteriological point of view.

REVIEW OF LITERATURE

Webb and Hall (13) found that fresh whole milk could be pasteurized, condensed to one-third its weight, canned, and frozen, without any detri-

[1] Published with the permission of the Director as technical article No. 203 of the Nebraska Agricultural Experiment Station.

[2] The data presented in this paper are from a study made by the junior author under the supervision of the senior author in partial fulfillment of the work required for the degree of Master of Science.

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mental effects to the body or flavor of the product. These investigators also found that skimmilk of 9 per cent solids was unchanged in heat stability (time required for heat coagulation at 120° C.) after storage for 33 weeks in a frozen condition whereas milk of 18 per cent solids did not possess any stability toward heat at the seventeenth week. Anderson and Pierce (1) showed that holding milk at −25.7° C. (−14° F.) gave no precipitated protein till the third month and was usable till the sixth month whereas milk held at −12.5° C. (10° F.) showed some protein precipitation at the end of the second month and at the end of the fifth month practically all protein had precipitated out. Doan and Baldwin (4), referring to the work of Webb and Hall, state that freezing, as such, has no measurable effect upon protein dispersion but rather that holding in a frozen condition, for several weeks or months, is required to definitely cause aggregation or instability of the proteins. Mack (5) found that the proteins in mixes made from frozen cream were less stable than those in mixes made from fresh cream, as measured by the protein stability test used by Doan (2). Ramsey (9) stated that the use of frozen condensed skimmilk in ice cream mixes to be used in continuous freezers, was unsatisfactory due to the denaturation of the proteins as a result of freezing. Ramsey, in a communication to the junior author (10), states that while the use of frozen condensed skimmilk in mixes to be used in batch freezing has proved fairly satisfactory, such mixes cannot be used in Vogt Freezers due to the poor quality of the ice cream produced. Doan (3) concluded that when frozen condensed whole milk is stored more than eight weeks, the proteins become denatured and a gelation occurs, when the milk is thawed.

EXPERIMENTAL METHODS

Preparation and Study of the Condensed Skimmilk

For the manufacture of the condensed skimmilk to be used in this study, skimmilk was taken immediately after separation from pasteurized whole milk and heated to a temperature of 170° F. for forewarming. It was then drawn into a Rogers 18-inch copper vacuum pan, where it was condensed to approximately one-fourth of the original weight, under a vacuum of about 23 inches. After testing for fat and solids, it was run into new five-gallon tinned lard cans and stored at approximately 0° F. The condensed skimmilk contained 36.76 per cent total solids and .32 per cent fat. A total of 9 cans was put into storage—one to be taken out each month, from September on through May.

As each monthly batch was withdrawn from storage and melted, by allowing to stand at room temperature for 48 hours or by setting in cold water, observations were made for possible changes in the physical structure of the condensed milk or presence of "off-flavors" that might have developed during the storage period.

The fresh condensed skimmilk used in each monthly batch was tested for solids and fat as soon as made. The mix containing this fresh condensed skimmilk was made up the same day and from identical ingredients in order to avoid variation in the composition.

Preparation of the Ice Cream Mixes

Once each month, for nine months, two 400 lb. batches of mix were made. One mix contained fresh condensed skimmilk as the concentrated source of serum solids while the other contained the condensed skimmilk that had been frozen and stored. Each mix was pasteurized at 150° F. for a period of thirty minutes, homogenized with a two-stage, 60-gallon per hour Colony homogenizer at a total of 3500 pounds per square inch pressure, with 500 pounds pressure on the second stage, and run over a surface cooler and cooled to approximately 40° F. The mix was run into 10-gallon milk cans and held below 40° F. till used.

The mixes were calculated to contain 14% butterfat, 10% serum solids, 15% sugar, .25% gelatin, and 39.25% total solids.

The ingredients were weighed carefully with the purpose of keeping the mixes as nearly alike as possible. In addition to the condensed milk used, the only other ingredients going into the mixes were fresh cream, fresh skimmilk, sugar, and gelatin.

Analytical Procedure

The butterfat and total solids content were determined by use of the Mojonnier method (7).

The pH of the ice cream mixes was determined by means of the Coleman pH electrometer, using a glass electrode (8), at 25° C. (77° F.). The titratable acidity determinations were made as outlined by Sommer (11).

Protein stability (2) of the samples was rated relatively, according to the least amount of alcohol (sp. gr. 0.8912 at 20° C.) required to produce the first noticeable trace of flocculation in 5 cc. of sample. In all cases water was added prior to the alcohol so that the total volume of the addition (water plus alcohol) amounted to 10 cc.

Method of Freezing

The ice cream mixes were frozen in a Creamery Package 40-quart horizontal brine freezer. The two comparable batches of mix which had been held at about 40° F. until used, were frozen, the mix containing the fresh condensed skimmilk always being first. A preliminary batch was always frozen in order to have the freezer cold and to whip each mix as nearly the same as possible. At the start of freezing 20 cc. of vanilla isolate extract were added to each batch of mix. The brine was shut off when the temperature was lowered to 22.6° F. Overrun readings were taken at minute intervals after the brine was shut off.

Method of Procuring Samples

Samples were obtained by filling quart Sealright containers—(two from each batch taken directly from the freezer) as soon as 100 per cent overrun was obtained, after which the remainder of the batch was run into parchment-lined five-gallon ice cream cans. The samples and cans were immediately transferred to the hardening room which was kept at a temperature of approximately 0° F.

Judging the Ice Cream and Determining Its Melting Quality

The quart samples were removed from the hardening room about two weeks after freezing and were cut in half. One pint of each quart was scored[3] for flavor and body. The remaining pint of the original quart sample was set upon a ¼-inch mesh wire resting on the rim of a 5-inch heavy glass funnel with the end leading into a 100 cc. graduate cylinder. The number of minutes it took for 100 cc. of the melted ice cream to collect in the cylinder was recorded for each sample. The character of the liquid in the cylinder and the amount of foam in the cylinder were also observed. Each batch was, therefore, represented by duplicate samples and the average of the two duplicates represented the figure taken for each sample.

EXPERIMENTAL RESULTS

Effect of Freezing and Storing upon the Milk Protein

During the nine months of the experiment, no change in appearance of the frozen condensed milk was noticeable, when it was first removed from the storage room. However, when it had been melted down it was observed that at the third month the condensed milk was coarser than when first made, and at the fourth month a partial gel had been formed. From the fifth month on, the milk had formed a complete gel and in addition, at the eighth and ninth months, some "wheying-off" was observed on thawing. The gel formation was readily broken up and dispersed when put into the vat as part of the mix and no trouble was experienced with it throughout the processing of the mix.

Table 1 shows that the number of cubic centimeters of alcohol required to give the first noticeable flocculation in each mix was comparatively constant all of the way through the experiment with the stability of the mixes containing the frozen condensed skimmilk consistently below that of the mixes containing the fresh condensed skimmilk. The amount of alcohol required to coagulate the frozen condensed mixes was 6.5 cc. for each determination except for the fourth, eighth and ninth months, when it was respectively 7.0 cc., 6.0 cc. and 6.0 cc. The fresh condensed mix always required 7.0 cc. to give coagulation.

[3] All samples were scored by Dr. P. A. Downs of the Department of Dairy Husbandry, University of Nebraska.

TABLE 1

Protein stability (to alcohol) of ice cream mixes containing fresh and frozen condensed skimmilk

Fresh		Frozen	
Mix No.	Alcohol No.*	Storage period in month	Alcohol No.*
1	7.0	1	6.5
2	7.0	2	6.5
3	7.0	3	6.5
4	7.0	4	7.0
5	7.0	5	6.5
6	7.0	6	6.5
7	7.0	7	6.5
8	7.0	8	6.0
9	7.0	9	6.0

* Alcohol number is the number of cc. alcohol required to give the first flocculation in 5 cc. of mix.

Effect of the Use of Frozen Condensed Skimmilk on the Acidity of the Ice Cream Mixes

In comparing the results of hydrogen-ion concentration determinations, no significant difference was observed between those of the mixes made using frozen condensed skimmilk stored for various intervals and those using fresh condensed skimmilk.

The titratable acidities varied slightly from batch to batch, and sometimes, from day to day as would be expected, but the differences were at no time large enough to be significant.

Effect of the Use of Frozen Condensed Skimmilk upon the Whipping Ability of Ice Cream Mixes

Table 2 shows overrun readings at minute intervals comparing the different batches of mix made from fresh and frozen condensed skimmilk over a nine months' period. These data are summarized in Graph 1 and show that at the end of one minute, the mix made from fresh condensed skimmilk was less in overrun, and this was continued in general, until between three and four minutes the overrun of both mixes became equal, being about 78 per cent. From that time on the conditions changed and the fresh condensed skimmilk mix showed the more rapid increase in overrun percentage, reaching its 100 per cent in eight minutes after the brine was shut off, whereas the mix containing frozen condensed skimmilk did not reach 100 per cent overrun until nine minutes after the brine was shut off. This difference was not large enough to warrant the condemnation of the use of frozen condensed skimmilk; it merely suggested that 100 per cent overrun is reached a little slower with frozen condensed milk than with fresh.

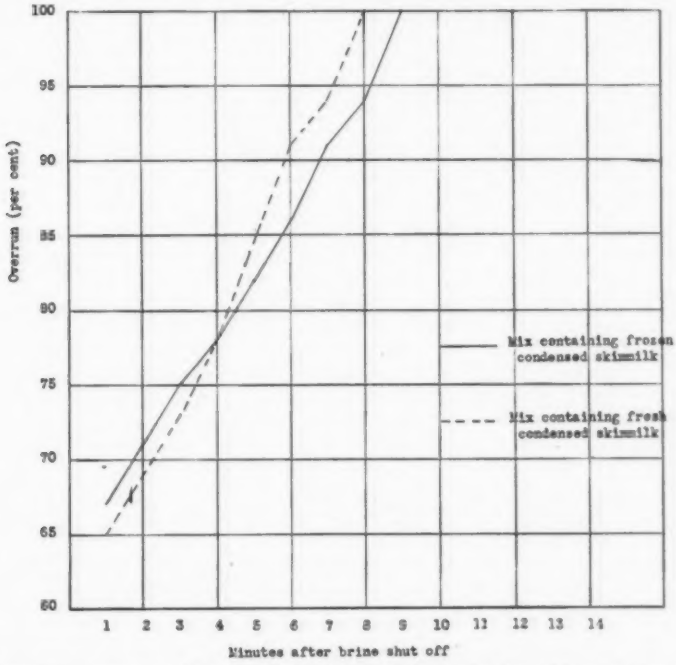
TABLE 2

Overrun freezing record of ice cream mix containing fresh and frozen condensed skimmilk

Mix No.	Condensed milk		Mix Temp. at freezer ° F.	Brine Temp. ° F.	Overrun readings at minute intervals after brine was shut off									
	Type of condensed milk used	Months in storage at 0° F.			1	2	3	4	5	6	7	8	9	10
1	Fresh	....	36	−4	70	72	75	80	85	88	92	100		
1a	Frozen	1	36	−2	73	75	77	80	82	85	88	94	100	
2	Fresh	....	36	−4	65	68	75	80	83	90	95	100		
2a	Frozen	2	36	−2	65	70	75	77	80	85	90	100		
3	Fresh	....	38	−6	60	65	70	70	75	85	90	100		
3a	Frozen	3	38	−4	60	65	68	75	80	85	86	90	100	
4	Fresh	....	38	−4	67	73	76	81	85	90	95	100		
4a	Frozen	4	38	−3	70	75	80	85	87	90	95	97	100	
5	Fresh	....	36	4	65	70	76	80	83	90	95	96	100	
5a	Frozen	5	36	6	67	72	75	77	80	85	88	92	95	100
6	Fresh	....	38	−10	65	70	73	80	84	90	94	95	100	
6a	Frozen	6	38	−8	66	70	75	79	82	84	89	92	96	100
7	Fresh	....	39	−2	60	65	70	73	78	85	95	100		
7a	Frozen	7	42	−2	56	62	63	65	70	75	82	85	92	100
8	Fresh	....	38	−2	60	65	70	75	80	86	93	100		
8a	Frozen	8	38	−2	60	65	70	77	82	90	92	97	100	
9	Fresh	....	36	−2	65	70	75	79	83	90	95	100		
9a	Frozen	9	36	0	60	62	66	70	75	80	85	89	95	100

Effect of the Use of Frozen Condensed Skimmilk upon Ice Cream Quality

The ice cream samples were all scored after having been in the hardening room about two weeks as it was believed that this would give most representative conditions. Table 3 shows that there was a noticeable difference in flavor in only three out of the nine comparative pairs of ice cream samples and only one of these samples was made from frozen condensed milk less



GRAPH 1. Overrun readings at minute intervals for 9 batches with each type mix.

than 6 months old. In that case, the sample was criticized for having a "Slight Condensed" flavor. From the seventh month on, the stored condensed skimmilk gave the ice cream samples an off-flavor which was described as "Condensed" or "Lacking in fine flavor." The comparative ice cream samples taken from the ninth month freezings were both given an equal cut in the flavor score.

From the standpoint of body and texture the nine pairs of ice cream samples made from frozen condensed milk were given a slightly lower score than the samples made from fresh condensed skimmilk. In two of the samples there was no difference in score while in the remaining case, the sample containing the fresh condensed skimmilk was given a lower score. "Iciness" to same degree was listed five times, "Coarseness" was listed twice as a

TABLE 3

Scores and criticisms of ice cream samples obtained from the two types of mixes

Type of condensed milk in mix	Age condensed milk	Scoring			
		Flavor		Body and texture	
		Criticism	Score	Criticism	Score
Fresh			45		24.0
Frozen	one month		45	coarse	23.5
Fresh			45	sl. fluffy	22.5
Frozen	two months		45	coarse	22.5
Fresh			45		24.0
Frozen	three mos.	sl. cond.	43	sl. icy	23.5
Fresh			45	sl. icy	23.0
Frozen	four mos.		45		23.5
Fresh			45		24.0
Frozen	five mos.		45	sl. icy	23.0
Fresh			45		24.0
Frozen	six mos.		45	icy	23.5
Fresh			45		24.0
Frozen	seven mos.	condensed	43	icy	23.5
Fresh			45		24.0
Frozen	eight mos.	lacks fine flavor	44		24.0
Fresh		lacks fine flavor	44		24.0
Frozen	nine mos.	lacks fine flavor	44	icy	23.5

Perfect Score—Flavor 50, Body and Texture 25.

defect in the samples containing the frozen condensed skim. The defects in no case were serious enough to warrant large cuts and under practical operating conditions such differences would not be noticed by anyone not skilled in judging ice cream.

Effect of the Use of Frozen Condensed Skimmilk upon the Melting Quality of Ice Cream

The ice cream samples were allowed to melt down at room temperatures; therefore the number of minutes required to collect 100 cc. of melted ice cream would be expected to vary from month to month. This occurred in the case of the samples containing the fresh condensed skimmilk as well as with the samples containing the frozen condensed skimmilk. Average results revealed that under identical conditions, the ice cream samples containing the frozen condensed skimmilk melted down twice as fast as the samples containing the fresh condensed skimmilk.

A possible explanation for this difference may be that the freezing and holding of condensed skimmilk alters the hydrophilic properties of the proteins and the inclusion of such condensed milk in an ice cream mix would alter somewhat the nature and degree of water holding capacity of the proteins in that mix. Sommer (12) states that the hydrated particles form a structure of filaments, with the free water being held in the capillary spaces between the filaments. The gel formed is due to the gelatin and milk proteins present; therefore where the influence of the gelatin was the same in

each case, the gel-forming ability is lessened when the degree of hydration of the milk proteins is lessened.

In every case it was observed that the melted ice cream made from mixes that contained frozen condensed skimmilk showed more foam than the melted ice cream containing the fresh condensed skimmilk.

DISCUSSION OF RESULTS

Our experiments have shown that frozen condensed skimmilk, after storage for four or more months, exhibited evidences of gelation upon being melted. This is evidence that some denaturation of the protein has taken place during storage. The poor appearance of the frozen condensed skimmilk upon melting might seem to hinder its use in ice cream making but the appearance of the final mix was not affected at all. When the thawed condensed milk was dumped into a vat as part of a mix, it was easily blended with the other ingredients, and no trouble was experienced in processing the mix.

No significant differences between the two types of mixes were found, as far as the pH and titratable acidity were concerned. Where slight differences were found, these could readily be explained by other factors, not dependent on differences in the condensed milks used.

The difference noted in whipping ability, between the two types of mixes, is small but might be large enough so that the use of frozen condensed skimmilk might not be desirable under certain plant conditions. The number of storage cans and the amount of storage space necessary would be a factor to be considered as to the economy of freezing and storing condensed skimmilk. It would appear that plants having a seasonal surplus of skimmiik would benefit most from such a plan, especially where the condensed product could be made on the premises. Adequate storage space held at a low temperature, is, of course, necessary.

Ice cream made from mixes containing frozen condensed skimmilk scored the same as ice cream made from the mixes made from the fresh condensed skimmilk in a majority of the comparisons made, as far as the flavor was concerned and scored slightly lower in body and texture.

The fact that the ice cream made from the mix that contained frozen condensed skimmilk melted down so much faster than the sample containing fresh condensed skimmilk should not be allowed to discourage any would-be user. It may be noted that these mixes contained only .25 per cent gelatin. If greater melting resistance is desirable increasing the gelatin content would probably produce it.

As frozen condensed skimmilk is usually held at cold storage temperatures (0° F.) and as a 5-gallon can of the frozen product takes from 48 to 60 hours to thaw, it would seem that in many instances frozen milk of this type may be shipped considerable distances without further refrigeration.

Frozen condensed milk has been shipped satisfactorily by the University creamery for distances of 150 miles in hot summer weather on non-refrigerated trucks.

SUMMARY AND CONCLUSIONS

1. The storage of frozen condensed skimmilk to be used in ice cream mixes for batch freezing is a satisfactory method of handling surplus skimmilk. A storage period exceeding six months does not appear advisable.

2. After four months in storage at 0° F., condensed skimmilk formed a gel when thawed, and after eight and nine months in storage a "wheying-off" was also observed.

3. No significant differences in pH and titratable acidity between mixes made with frozen condensed skimmilk and fresh condensed skimmilk were recorded.

4. The whipping ability of mixes containing frozen condensed skimmilk was somewhat less than that possessed by mixes containing fresh condensed skimmilk. A slightly longer time was required in the freezer to reach a definite percentage of overrun. No attempts were made to reach a maximum overrun reading.

5. The use of frozen condensed skimmilk in the ice cream mixes caused a lower flavor score in three of the nine trials. The body and texture score was lower in six out of nine cases where frozen condensed skim was used. Neither differences were very significant or consistent.

6. With the gelatin content held constant in each mix, the mix containing frozen condensed skimmilk produced an ice cream that melted down approximately twice as fast as the ice cream made from a mix containing fresh condensed skimmilk.

7. The frozen condensed skimmilk when removed from storage may be shipped considerable distances under comparatively high temperatures with no additional refrigeration necessary.

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RELATION OF COLOR AND ASCORBIC ACID TO FLAVOR IN MILK FROM INDIVIDUAL COWS[1]

O. F. GARRETT, H. H. TUCKER, AND F. C. BUTTON
New Jersey Agricultural Experiment Station, New Brunswick, N. J.

In 1935, Chilson (4) reported that vitamin C (ascorbic acid) exerted a pronounced influence in preventing the development of oxidized or tallowy flavor in milk when it was added directly to milk. Sharp, Trout and Guthrie (6) later studied this problem and reported that, "There is a positive correlation between the rate of oxidation of ascorbic acid and the rate of development of the oxidized flavor." Brown, Thurston and Dustman (3) found that, "Dry feeding increased the tendency for oxidized flavor to develop in milk, and grazing on fresh pasture decreased this tendency." These latter findings are corroborated by experiments carried out at the New Jersey Agricultural Experiment Station (5). Although it is generally believed that vitamin C in milk cannot be greatly increased by feeding, Brown, Thurston and Dustman (3) found that the feeding of tomato or lemon juice or pure crystalline ascorbic acid greatly decreased the tendency for oxidized flavor to develop even when the cows were on dry feed.

Anderson (1, 2), in collaboration with Harbenbergh and Wilson, has reported that "all available data concerning feeds strongly suggest a relation between carotene and ability to produce milk of good flavor."

In an experiment conducted at the Sussex branch of the New Jersey Agricultural Experiment Station from December 1, 1936, to April 12, 1937, the following factors were studied:

1. The relation of the percentage of fat to yellow color of milk.
2. The relation of yellow color to ascorbic acid in milk.
3. The relation of yellow color to flavor of milk.
4. The relation of ascorbic acid to flavor of milk.

A total of 28 cows consisting of 16 pure bred Guernseys and 12 pure bred Holsteins were used in this study which was carried over a period of approximately four months. The first samples were taken on January 7, 1937, and the last ones on April 29, 1937. Results on samples taken on February 4th and February 11th are not presented in this study since the milks were inadvertently exposed to bright sunlight before reaching the laboratory.

Individual samples from single milkings were taken at weekly intervals and were immediately analyzed for fat by the Babcock method and for

[1] Recently it has been indicated that dehydroascorbic acid shows vitamin C potency. Since the dye-titration method used in this study does not determine the dehydro form the term "ascorbic acid" is used instead of the term "vitamin C."

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yellow color with the "lactochrometer."[2] Each sample was divided into three parts, the last two of which were stored in glass bottles in a refrigerator at about 40° F. The first part of each sample was analyzed for ascorbic acid by the dye-titration method and immediately thereafter was scored for flavor. The second and third parts of each sample were removed from storage on the two succeeding days respectively and were also analyzed for ascorbic acid and scored for flavor.

TABLE 1

Showing the rank of percentage of fat, flavor, ascorbic acid, and color

Cow No.	Per cent fat* rank	Flavor rank*		Ascorbic acid rank*		Color* rank
		First day	Third day	First day	Third day	
470 G	6	1	8	9	5	2
428 G	19	2.5	2	14	12	9.5
471 G	8	2.5	4	13	15	7
267 G	7	5	5	4	4	3
467 G	2	5	6.5	21	24	8
479 G	3	5	9.5	3	10	17
179 G	5	7.5	1	8	9	5
465 G	1	7.5	9.5	1	1	1
78 H	23	9	19.5	5	3	20
474 G	12	11.5	6.5	17	19	14
273 G	10	11.5	11.5	2	2	6
422 G	9	11.5	13	10	11	11
165 G	4	11.5	14	6	6	4
92 H	17	14	15	16	14	18
484 G	11	15	21	22	20	13
215 H	13	16.5	3	11	8	15
478 G	15	16.5	16	7	7	12
217 H	28	18	9.5	19	18	24
229 H	18	19.5	17.5	12	13	22
276 H	24	19.5	17.5	20	16.5	19
253 H	20	21	22	18	16.5	23
234 H	27	22	19.5	26	25	28
284 H	25	23	24.5	27	22	26
230 H	26	25	23	23	21	27
286 H	21	25	26	24	26	21
431 G	13	25	27	15	23	9.5
220 H	22	27	24.5	25	27	25
285 G	15	28	28	28	28	16

G—Guernsey; H—Holstein.

* The ranking of each factor is based on the average for each cow throughout the experiment for that particular factor.

In studying the relation between color, fat, ascorbic acid and flavor statistical analyses of the results were made. The coefficients of correlation were obtained in all cases by Spearman's rank-difference method. A correlation coefficient of .4 or better was considered significant.

[2] The "Lactochrometer" is a modification of the Pfund instrument devised by the Department of Dairy Husbandry of the New Jersey College of Agriculture in cooperation with the American Guernsey Cattle Club for the rapid determination of yellow color in milk from all sources.

TABLE 2

Correlations

Variables	Correlation coefficient
1. Color vs. Percentage of fat	
a. All samples	+.8702
b. Guernseys	+.5317
c. Holsteins	+.7366
2. Color vs. First day ascorbic acid	+.6847
3. Color vs. First day flavor	
a. All samples	+.7339
b. Guernseys	+.4834
c. Holsteins	+.7229
4. Color vs. Third day flavor	+.6039
5. First day ascorbic acid vs. First day flavor	+.6996
6. First day ascorbic acid vs. Third day flavor	+.5779
7. Third day ascorbic acid vs. Third day flavor	+.5843

RESULTS

There was a close relationship between the percentage of fat and the color of milk, Tables 1 and 2. This is shown by a correlation coefficient of +.8702 between these two variables when the data from all 28 cows were used in the computation. When the data were segregated as to breeds the correlation coefficients were +.5317 and +.7366 respectively for Guernseys and Holsteins. This indicates that the percentage of fat had a more positive effect on the color of the Holstein milk than it had on the Guernsey milk.

The correlation coefficient of +.6847 between color and first day ascorbic acid (ascorbic acid determined on the day of the milking) indicates a very significant relationship between these two variables. It is quite possible that both factors are not only closely related to feeds and feeding practices but also may depend alike on certain hereditary factors of the individual cow and on the physiological condition of the cow.

The yellow color of milk is primarily due to the yellow pigment, carotene. A close inspection of the molecular structure and the reactions of carotene reveals that it has reducing properties. It is known that the presence of reducing substances in milk will tend to prevent an oxidizing reaction in the milk. It would be expected, therefore, that carotene would exert a protective action on the flavor of milk. That a close positive relationship exists is shown by correlation coefficients of +.7339 between color and first day flavor and +6039 between color (first day) and third day flavor (scored after about 56 hours' storage). These two coefficients indicate that, while the relationships between the two factors may be partly coincidental, color or carotene does help to stabilize flavor. This is especially indicated by the correlation between color and flavor after the milk has been in storage for some considerable time.

Ever since the molecular structure of ascorbic acid was discovered, it has been known that the compound has reducing properties. Accordingly

TABLE 3

Distribution of flavor scores in ascorbic acid classes

Ascorbic acid class intervals, mg./l.	Flavor score class intervals								Number cases in each class	Average score for each class	Per cent of cases with score of 23	Per cent of cases with score below 21.5
	23	22.5	22	21.5	21	20.5	20	19.5–12				
30												
27	3	1							4	22.88	75.0	0
24	4		1						5	22.80	80.0	0
21	29	8	2						39	22.85	74.4	0
18	62	24	10	4					100	22.72	62.0	0
15	138	30	14	13	4	1	2		202	22.68	68.3	3.5
12	121	48	36	14	8	1	6	3	237	22.42	51.1	7.2
9	92	58	26	25	11		12	6	230	22.20	40.0	12.6
6	34	19	12	7	11	2	5	10	100	21.73	34.0	28.0
3	11	12	8	5	5		2	5	48	21.45	22.9	25.0
0	2	1	6	1	3		1	2	16	21.34	12.5	37.5
Totals	496	202	114	69	42	4	28	26	981		50.6	10.2

it can be predicted that ascorbic acid should exert a protective action on the flavor of milk similar to that shown by carotene. A statistical analysis of the data from all 28 cows shows that such a relationship does exist. The correlation coefficient of +.6996 between first day ascorbic acid and first day flavor is quite significant, while significant coefficients between first day ascorbic acid and third day flavor and between third day ascorbic acid and third day flavor indicate that ascorbic acid helps to stabilize flavor. This relationship is further shown in Table 3. In making this table the ascorbic acid values were divided into three-milligram intervals; the flavor scores were divided into 0.5-point intervals. In plotting the distribution, only data from single samples were used; no averages appear in this table. There was a total of 981 samples on which the flavor score and ascorbic acid content were obtained. An inspection of the average flavor score for each ascorbic acid class interval reveals that with a decrease in the amount of ascorbic acid a decrease in the flavor score occurs. A further inspection of the data reveals that an apparent critical point in the relation of ascorbic acid to good flavor lies somewhere between 15 and 18 milligrams of ascorbic acid per liter of milk.

CONCLUSIONS

From the data presented in this paper it can be concluded that in the milk from individual cows:

1. There is a close relation between percentage of fat and yellow color.
2. There is a significant association of yellow color and ascorbic acid.
3. High carotene and high ascorbic acid are coincidental to and help to preserve good flavor in milk.

It is recommended, therefore, that special efforts be made to preserve the carotene in roughages and that winter feeding practices be followed so as to maintain a high level of yellow color in the milk.

It is further recommended that special efforts be made to preserve the ascorbic acid in milk by subjecting the milk to a minimum amount of aeration, by prevention of contamination with such metals as copper, iron and nickel, and by protection of the milk from sunlight.

ACKNOWLEDGMENT

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RELATION BETWEEN LACTOSE AND ASH CONTENT OF THE MILK OF DIFFERENT MAMMALS

PAUL F. SHARP

Department of Dairy Industry, Cornell University, Ithaca, New York

The freezing-point depressions of the milk and blood of cows are essentially the same. The freezing-point depression of the milk is almost entirely due to lactose and salts. Milk from diseased udders or from cows in ad-

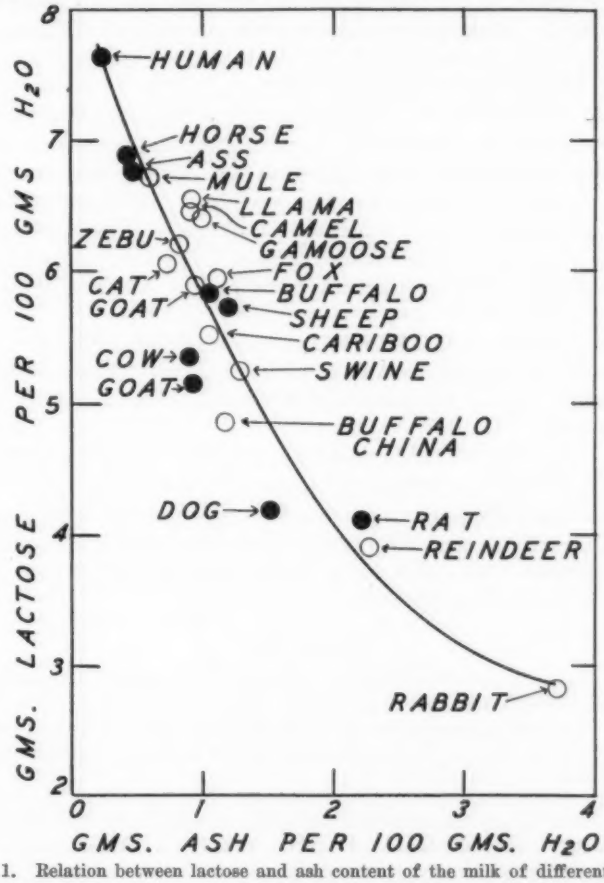

FIG. 1. Relation between lactose and ash content of the milk of different mammals when calculated to the water basis. Circles represent few data available; dots represent more extensive data.

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vanced lactation is relatively low in lactose, but this is compensated for by a higher salt content, the freezing-point depression remaining essentially unchanged (1, 2, 3). These alterations, particularly the increase in chloride content (4), are used for detecting abnormal milk. Since the blood and milk are essentially in osmotic equilibrium and the freezing point of the blood of the different mammals is much the same, one would expect the osmotic pressures of their milks to be about the same.

The milk of different mammals for which apparently reliable data on composition could be found ranged in average values for water from about 65 to 88 per cent; for ash from 0.23 to 2.5 per cent; and in lactose from 2.0 to 6.7 per cent. These wide variations are brought into a definite relationship to each other when calculated on the basis of the amount of water in the milk. This relation is shown in Figure 1.

The data were taken from compilations (5) and many individual articles. Data were not included when it was obvious that either the milk was abnormal or the values were in error. Some variation was to be expected because of the differences in analytical methods. The insolubility of some of the ash constituents, the presence of simple organic compounds, and the variation in the percentage composition of the ash constituents might exert an influence, but these variations (5) apparently affect the general relationship to only a minor degree.

The data found for the aquatic mammals, the elephant, and the hippopotamus were not included because of inconsistencies. These inconsistencies were probably due to the difficulties in obtaining normal, representative samples of milk.

The inverse relationship between lactose and ash constituents has long been recognized in the variations occurring in the milk of the cow. Figure 1 shows that in general the same inverse relationship holds when applied to the milk of different mammals when the calculations are made on the basis of water content. The curve serves as a rough criterion for the reliability of lactose and ash values for milk.

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A METHOD OF COUNTING VIABLE BACTERIA IN MILK BY MEANS OF THE MICROSCOPE[1]

GEORGES KNAYSI AND MARK FORD

Department of Dairy Industry, Cornell University, Ithaca, New York

INTRODUCTION

The value to the dairy industry of a microscopic method which allows distinction between dead and living bacterial cells needs no emphasis. Heretofore, the difficulties in the way of such a method seemed insurmountable. The two attempts we were able to find in the literature are those of Beattie (1) and Nadelin (13) which are really applications of Proca's method (14) and of its modification by Kayser (7). Failure of these attempts was to be expected because of defects inherent in the methods of Proca and Kayser. Knaysi's criticism (8) of these methods has been confirmed again in the present investigation.

PRELIMINARY EXPERIMENTS

Before the writers undertook the present investigation, they were fully aware of the magnitude of the task. They found it necessary to approach the problem with open minds and to avail themselves of the published experiences of other investigators who concerned themselves with the problem of microscopically distinguishing dead from living cells. Accordingly, we tried, but without success, Proca's method as used by Beattie and in the form of Kayser's modification. We were also unsuccessful when we used Růžička's method (15), Benians' method (2) and its modification by Henrici (6), Drobot'ko's method (3), and an adaptation of the recent one suggested by Luyet (11). In the latter case, we attempted several other modifications of our own, but got nowhere. The only application which seemed to hold promise for us was that of "vital staining" with basic dyes as used by the senior author (1935).

Difficulties of Application

It has been shown by Knaysi (8) that the slightest shade of staining of the cytoplasm indicates death of the cell, while the cytoplasm of a viable cell is perfectly colorless. With basic dyes the process of staining is instantaneous and is not dependent on autolysis; on the contrary, autolysis reduces stainability with basic dyes. Our problem was, therefore, to make such a method applicable to milk. In this case it is not sufficient for the

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method to be scientifically correct; it must be, in addition, simple, practical and, if we may say so, "fool-proof," before it can be suitable for the routine work of milk control. It was, therefore, necessary to make several changes in the technique used by the senior author, which consisted in mixing measured volumes of culture and neutral red containing gelatin, and counting in a Petroff-Hauser chamber after cooling the chamber in an icebox to cause hardening of the gelatin.

In the first place, the Petroff-Hauser chamber is expensive; secondly, room temperature is often too warm and one would have to work in a cool room to keep the gelatin hard; and thirdly, neutral red, which was selected for the theoretical study on account of its low characteristic potential, is not the best dye to use because of its color, its sensitivity to pH and its ability to stain fat with a yellow tinge. Moreover, milk contains an immense number of minute fat droplets which, when singly or in pairs are easily confusable with viable coccus cells.

Development of the Technique

Knaysi and Naghski (9), in an unpublished work, had substituted for the counting chamber the following technique: 0.01 cc. of the milk-gelatin mixture is placed on a clean slide and a clean cover glass is dropped over the droplet formed. The droplet is thus flattened into a film between the slide and the cover glass, which occupies an area equal to that of the face of the cover glass. This area, being known, a factor can be computed. In our work, we have adhered to this technique; however, in order to keep the factor down, we selected the smallest cover glass available to us, a square one, with 1.5 cm. to the edge. This gives, when used with an oil immersion objective and a $10\times$ ocular (10×97 combination), a factor of about 2.25×10^6. Using a $6\times$ ocular, the factor is reduced to about 1.35×10^6. In a few of our later experiments we cut our cover glasses down to 1.28×1.28 cm., thus having a factor of 1×10^6 when the 6×97 combination is used.

The next step was to modify the hardening agent. For this purpose we made several determinations of the "melting" and "solidification" points under practical laboratory conditions, of some agar and gelatin gels and of mixtures of the two when added to milk. On the basis of the results obtained, we picked a mixture which, when added to the milk, gives a percentage of 0.5 per cent agar and 2 per cent gelatin. Milk containing these percentages of agar and gelatin usually hardens in a few minutes at 23°–24° C. and once hard, has to be heated to about 70° C. before it melts.

Instead of neutral red, we used Nile blue sulfate, alone or mixed with methylene blue. The blue color gives a greater contrast, and the fat droplets take up an orange-red color which helps to set them apart. Therefore, when using such a mixture, the dead bacteria appear blue, the viable ones remain colorless, and the fat droplets assume various shades of red.

The Removal of Fat

Milk contains a large number of fat droplets many of which are within the size range of bacterial cells. It is these droplets that are most troublesome because as it was pointed out above, they can frequently be confused with viable coccus cells; attempt was therefore made to remove the fat. Unfortunately, it was found that the smallest fat droplets cannot be removed by ordinary centrifuging, and we found it necessary to think of removing the fat chemically without killing the bacteria in the milk. For that purpose, we experimented with several fat solvents, including hdyrocarbons of the methane series.

The experiments were carried out as follows: Sterile skimmilk was inoculated with a given organism and incubated at 37° C.; when sufficient growth had taken place, one cc. was measured into a water blank for plating, and the fat solvent was then added to the rest of the culture and the tube shaken vigorously for about two minutes. The shaken mixture was then allowed to stand for five minutes, after which it was centrifuged to remove the solvent droplets distributed throughout the tube. In the meantime, plating of the cc. first measured was carried out. After centrifuging, the culture treated with the fat solvent was also plated. Tables 1 and 2 contain the

TABLE 1

Effect of shaking with pentane and hexane

Organism	Plate counts		
	Before treatment	After treatment with pentane	After treatment with hexane
Streptococcus lactis	182,000,000	165,000,000	209,000,000
Streptococcus mastitidis	536,000,000	504,000,000	302,000,000
Escherichia coli	221,000,000	260,000	27,000,000
Proteus vulgaris	153,000,000	20,000	8,000,000
Unidentified organism	319,000,000	252,000,000	274,000,000

TABLE 2

Effect of shaking with hexane and heptane

Organism	Plate counts		
	Before treatment	After treatment with hexane	After treatment with heptane
Streptococcus lactis	61,000,000	58,000,000	59,000,000
Escherichia coli	39,200,000	36,000,000	67,000,000
Aerobacter aerogenes	4,800,000	1,900,000	5,400,000
Proteus vulgaris	61,000,000	27,000,000	109,000,000
Unidentified organism	33,000,000	7,000,000	11,000,000

results obtained with pentane, hexane and heptane. These results are in agreement with those of Hall (4), Walbum (17), and Morel, Rochaix and Mathais (12) regarding the toxicity of hydrocarbons.

Since heptane showed practically no toxic power toward the organisms employed, it was adopted for use in this work whenever fat removal was desired. However, when the final technique was developed, it was found that fat removal can be dispensed with.

We wish also to report at this point certain experiments in which we sought to remove the milk fat by agglutination and subsequent centrifuging. In an unpublished work, Krukovski and Sharp (10), of this department, discovered in milk an agglutinin for the fat droplets. On account of a specific protein film surrounding fat droplets in milk, we sought to develop agglutinins for these droplets, by injecting cream, washed free from casein, into guinea pigs. Two guinea pigs received daily intraperitoneal injections with this washed cream. After six days, the pigs were allowed to rest for six more days, after which they were bled from the heart and their serum added to milk in various concentrations. Definite agglutination of the fat droplets was observed in dilutions of 10^{-2} and below at 35° C. However, the process was found slow and unsuitable, and the removal of fat incomplete. We wish here to express our thanks to Dr. F. R. Smith for his technical help in this phase of the work.

FINAL PROCEDURE

After considerable testing and re-testing of steps and materials and the working out of many details, we standardized our procedure as follows:

1. One cc. of the well mixed sample is measured into each of two clean test tubes, A and B.

2. To each test tube is added 0.5 cc. of a dye mixture containing equal parts of 0.2 per cent methylene blue and 0.2 per cent Nile blue sulfate, and the contents of each tube mixed well. The final concentration of each dye in the completed preparation will be 0.025 per cent. Table 3 shows that in

TABLE 3

Effect of the dyes on the growth of milk cultures

10 cc. of culture was diluted with 5 cc. of sterile distilled water or with 5 cc. of a dye solution containing 0.1 per cent of Nile blue sulfate and 0.1 per cent of methylene blue. Plate counts were then made at intervals.

Time in min.	Streptococcus lactis plate count per cc. when diluted with		Aerobacter aerogenes plate count when diluted with	
	Sterile water	Dye solution	Sterile water	Dye solution
0	31,000,000	35,000,000	29,000,000	20,000,000
10	46,000,000	29,000,000	24,000,000	17,000,000
20	35,000,000	28,000,000	31,000,000	24,000,000
30	42,000,000	24,000,000	29,000,000	20,000,000
40	29,000,000	30,000,000	33,000,000	31,000,000
50	32,000,000	32,000,000	32,000,000	21,000,000
60	42,000,000	31,000,000	33,000,000	29,000,000
80	44,000,000	34,000,000	49,000,000	42,000,000
120			76,000,000	62,000,000

such a concentration these dyes are not toxic to *Streptococcus lactis* and *Aerobacter aerogenes*.

3. To one of the tubes, say tube B, add a drop of 0.7–0.8 N NaOH and mix. Tube A receives nothing.

4. To each tube add 0.5 cc. of the melted and tempered hardening agent which is an agar-gelatin mixture containing 2.0 per cent agar and 8.0 per cent gelatin and mix the contents of each tube. Before this is done, the tubes are placed for a few moments in the water bath alongside the melted agar-gelatin mixture. The temperature of the bath should be about 45°–50° C. This prevents the instantaneous hardening of the mixture. The hardening agent is now present in the concentration referred to above, namely, 0.5 per cent agar and 2.0 per cent gelatin.

5. With a Breed pipette, measure out 0.01 cc. from tube A and place on the center of the right half of a clean glass slide, and immediately cover the droplet thus formed with a clean square cover glass 1.28×1.28 cm. The droplet spreads and forms a film occupying the space between the cover glass and the slide.

Do the same with tube B, placing the 0.01 cc. droplet on the center of the left half of the slide, etc.

6. Let the preparation stand or place it in the icebox for about two minutes so that the films harden.

7. Count the blue-stained bacterial cells in a certain number of fields of each film and calculate the bacterial content of each per cc. With a 6×97 combination, and using 1.28×1.28 cm. cover glass, the factor is about 1,000,000.

The stained cells from tube A represent the dead cells present originally in the milk. The stained cells from tube B represent the total bacterial content of the milk. The difference represents the viable cells present in the milk.

With ordinary market milk we found it in general unnecessary to remove the fat. If it is desired to remove the fat, the milk sample should be warmed to 35° C. to liquefy the fat; heptane is then added and shaken vigorously for 3 to 5 minutes and centrifuged. A Babcock hand or electrically driven centrifuge is sufficient. The hydrocarbon-fat layer is then removed. A convenient way of removing this layer is by suction with a capillary glass tube attached to a water pump.

Even after this treatment, a certain number of the minute fat droplets remain. These show only a trace of orange color and could be confused with colorless cocci. This is one of the reasons why we finally modified our technique to the double-counting procedure outlined above. Moreover, we find it hard on the eyes to hunt for colorless cells, and easy to overlook many of them. This explains the low counts of Table 4. In the above procedure all the cells counted are stained deep blue against an almost colorless back-

TABLE 4

A comparison of counts obtained by the single count technique (living cells unstained) and by the petri plate method

Experiment No.	Culture employed	Dead cells $\times 10^6$	Group count $\times 10^6$	Total cell count $\times 10^6$	Plate count $\times 10^6$
5	*Streptococcus thermophilus*	0	17.8	28.1	40.0
8	*Streptococcus fecalis*	11.1	28.6	43.0	280.0
16	*Aerobacter aerogenes*	0	10.6	14.0	66.0
20	Unidentified culture	0	10.8	14.0	71.0
27	*Aerobacter aerogenes*	4.7	11.7	20.2	36.0
40	*Aerobacter aerogenes*	.5	6.7	21.4	20.0
48	*Aerobacter aerogenes*	4.2	6.8	11.7	27.8
54	*Aerobacter aerogenes*	12.8	13.7	15.5	35.0
59	*Aerobacter aerogenes*	7.2	6.3	8.1	49.0

ground. Even the presence of the fat droplets does not materially interfere with the visibility, mainly because in this method the fat droplets are mostly distributed throughout the film, and do not accumulate into a layer on the top of the film.

Calculation of the Factor

The procedure followed in computing the factor is similar to the one used in Breed's method. To illustrate, we propose to find the factor when 1.28×1.28 cm. cover glass and when a combination is used that gives a field diameter of 160 μ. The area of the field will be πr^2 or $3.1416 \times 0.008 \times 0.008 = 3.1416 \times 0.000064$ sq. cm. or about 0.0002 sq. cm.

The area of the preparation is $1.28 \times 1.28 = 1.64$ sq. cm., which is equivalent to $1.64 : 0.0002 = 8200$ fields, and contains 0.005 cc. of milk. The volume under each field would hold $.005 : 8200 = \frac{5}{8{,}200{,}000}$ cc. milk. The factor would, therefore, be 1,640,000. Roughly with the same combination, the factor is nearly three times that of the Breed smears.

APPLICATION OF THE METHOD

The method was applied to milk cultures of a number of organisms and to pasteurized market milk that in some cases had been allowed to incubate until it developed a high count. The results are recorded in Tables 5 to 9. For the sake of comparison, petri plate counts were included in each experiment, and are recorded in the tables. Although counts by our method did

TABLE 5

A comparison of differential microscopic counts and plate counts Streptococcus thermophilus

Experiment	Dead cells $\times 10^6$	Total count		Living cells $\times 10^{6*}$	Plate count $\times 10^6$
		Groups $\times 10^6$	Individuals $\times 10^6$		
1	4.5	9.0	18.0	13.5	8.0
2	3.0	5.8	11.7	8.7	Spoiled
3	2.0	6.7	21.1	19.1	Spoiled
4	7.0	4.8	13.5	6.5	4.6
5	5.1	5.2	10.0	4.9	3.9
6	2.7	4.0	10.7	8.0	5.6
7	0.9	4.2	13.2	12.3	9.0
8	0.55	3.15	9.7	9.15	10.0
9	0.70	8.0	19.1	17.4	13.0
10	0.45	4.0	10.3	9.85	7.3
11	0.23	1.5	3.8	3.57	2.9
12	0.34	1.25	4.5	4.16	3.6
13	0.22	1.12	3.37	3.15	1.2
14	0.7	5.8	27.7	27.0	17.0
15	1.0	6.0	36.45	35.45	13.0
16	1.13	10.1	23.3	22.17	41.0
17	0.34	4.5	10.7	10.36	7.6
18	0.78	5.1	12.8	12.02	9.0
19	0.56	13.2	34.9	34.34	24.0
20	0.45	3.3	9.4	8.95	6.0
21	0.45	8.4	23.1	22.65	15.0
22	0.45	8.7	24.1	23.65	20.0
23	1.10	9.2	25.4	24.3	12.0
24	0.6	7.3	24.6	24.0	20.0
25	0.45	11.2	31.0	30.55	25.0
26	0.6	6.1	20.25	19.65	13.0
27	0.78	7.9	28.7	27.92	12.0
28	0.35	2.0	7.1	6.75	4.1
29	1.90	13.3	41.2	39.3	27.2
30	0.90	5.1	19.5	18.6	12.6
31	0.23	1.79	6.2	5.97	2.0
32	0.67	1.69	4.9	4.23	1.8
33	0.30	1.24	3.3	3.0	1.4
34	0.45	1.35	6.5	6.05	Spoiled
35	0.56	1.8	5.4	4.84	7.0
36	0.67	3.6	8.3	7.63	7.7

* Total individuals minus dead cells.

not include viable groups and cannot strictly be compared to the plate counts, yet we find the great majority of the plate counts occupying the range between the total group and viable individual counts of our method. Of interest are the experiments made on the pasteurized milk. The majority of the plate counts fall below the total group count, which is often higher than the number of viable cells. These results are what one would expect from pasteurized milk.

Stainability of Cells Killed by Pasteurization

The stainability of cells killed by pasteurization has been investigated by a number of workers. Hastings and Davenport (5) concluded that the

TABLE 6

A comparison of differential microscopic counts and plate counts Streptococcus lactis

Experiment	Dead cells $\times 10^6$	Total count		Living cells $\times 10^{6*}$	Plate count $\times 10^6$
		Groups $\times 10^3$	Individuals $\times 10^6$		
1	0.70	21.6	82.3	81.6	37.0
2	0.56	17.3	53.1	52.54	35.0
3	1.2	13.3	38.0	36.8	29.0
4	1.2	15.3	48.1	46.9	36.0
5	0.75	13.5	32.2	31.45	22.0
6	0.9	10.6	21.8	20.9	25.0
7	0.9	8.1	21.2	20.3	20.0
8	0.45	9.5	20.9	20.45	27.0
9	0.15	9.6	28.1	27.95	30.0
10	0.56	2.3	5.1	4.54	4.2
11	0.9	6.4	12.8	11.9	10.0
12	0.25	3.3	7.2	6.95	6.5
13	0.34	1.13	2.25	1.91	2.8
14	1.4	7.1	17.1	15.7	17.0
15	2.0	3.0	4.7	2.7	Spoiled
16	1.1	2.4	3.4	2.3	3.0
17	1.35	4.15	8.0	6.65	8.0
18	0.9	5.8	14.3	13.4	13.5
19	0.67	8.3	15.2	14.5	13.0
20	0.45	6.5	13.7	13.25	12.5
21	1.0	1.3	2.7	1.7	.9
22	0.23	1.24	1.69	1.46	1.0
23	0.34	0.79	1.35	1.0	.8
24	0.11	0.61	1.05	0.94	1.0
25	0.10	4.5	14.6	14.5	15.0
26	1.1	5.3	18.35	17.25	8.2
27	0.23	1.47	2.9	2.67	3.9
28	0.56	3.15	7.9	7.34	5.3
29	0.67	1.45	1.8	1.13	0.9
30	0.34	1.8	3.5	3.16	2.8
31	0.11	1.1	1.8	1.69	2.6
32	0.11	2.15	3.8	3.69	5.6
33	0.56	1.8	3.7	3.14	2.2
34	0.45	2.0	3.05	2.6	2.5

* Total individuals minus dead cells.

number of bacteria demonstrable in milk after pasteurization by Breed's method varies from 3 to 83 per cent of the number demonstrated in the same milk before pasteurization, and depends considerably on the type of bacteria present. The results obtained by Ward and Myers (18) with pasteurized milk are similar, but these authors did not study the effect of the type of organisms killed by pasteurization. The conclusion drawn by Hastings and Davenport was that the direct microscopic method does not indicate the quality of the milk before pasteurization. On the other hand, Ward and Myers conclude "that insufficient numbers of dead bacteria remain visible after pasteurization to impair the usefulness of direct microscopic counts made on pasteurized milk." The latter authors, therefore,

TABLE 7

A comparison of differential microscopic counts and plate counts
Bacillus subtilis

Experiment	Dead count total cells $\times 10^6$	Total count		Living cells $\times 10^{6*}$	Plate count $\times 10^6$
		Groups $\times 10^6$	Individuals $\times 10^6$		
1	0.65	1.13	3.15	2.50	0.3
2	0.23	0.56	2.25	2.02	0.4
3	0.45	1.2	3.4	2.95	1.4
4	0.80	2.5	7.3	6.5	2.5
5	0.12	.81	2.0	1.88	1.4
6	0.34	1.8	4.4	4.06	3.1
7	0.23	1.35	2.9	2.67	1.8
8	0.12	4.0	8.2	8.08	5.0
9	0.12	2.6	4.8	4.68	2.9
10	0.23	2.4	3.8	3.57	5.1
11	0.34	1.8	3.8	3.46	2.9

* Total individuals minus dead cells.

TABLE 8

A comparison of differential microscopic counts and plate counts
Staphylococcus albus

Experiment	Dead cells $\times 10^6$	Total count		Living cells $\times 10^{6*}$	Plate count $\times 10^6$
		Groups $\times 10^6$	Individuals $\times 10^6$		
1	0.45	4.6	8.4	7.95	4.6
2	0.25	3.15	4.6	4.35	3.0
3			Spoiled		1.2
4	1.7	7.3	20.7	19.0	9.1
5	0	1.0	2.6	2.6	2.3
6	0.11	4.05	8.9	8.79	10.3
7	0.34	1.8	5.3	4.96	3.6
8†	0.4	2.5	9.35	8.95	8.1
9†	0.14	2.15	6.20	6.06	6.3
10†	0.2	3.9	7.8	7.6	10.5

* Total individuals minus dead cells.
† Counts made on whole milk without fat removal and using the 6× ocular.

consider counts obtained by the direct microscopic method to represent viable cells.

While we have not yet done sufficient work on this point to justify our taking a stand, we wish to report some preliminary results we obtained with milk and with pure cultures of *Streptococcus lactis*. The milk, or the culture was held at 63°–65° C. for 30 minutes, and immediately afterwards a count was made by our method and, in the case of milk, by the method of Breed, using Newman's solution no. 2 for staining. The results are recorded in Tables 10 and 11. These results show that the pasteurization of milk does not render the killed bacteria invisible when our method is used. On the other hand, we find confirmation of the earlier reports that pasteuri-

TABLE 9

Comparison of differential microscopic counts and plate counts
Commercial pasteurized milk

Experiment	Dead count total cells	Total count		Living cells $\times 10^{6*}$	Plate count $\times 10^6$
		Groups $\times 10^6$	Individuals $\times 10^6$		
1†	1.0	1.57	3.4	2.4	2.42
2†	1.0	1.01	1.35	0.35	.011
3†	1.125	1.8	3.4	2.275	0.80
4†	1.8	2.0	2.47	0.67	0.49
5†	1.35	1.7	1.90	0.55	0.47
6	1.1	1.8	2.0	0.9	0.61
7	1.57	16.2	25.0	23.4	36.0
8	8.65	9.35	12.4	3.75	2.0
9	2.25	12.25	18.75	17.5	13.0
10	0.34	0.27	0.42	0.08	0.01
11	0.21	0.54	1.01	0.8	0.30
12‡	0.45	3.65	6.2	5.75	6.1
13‡	1.75	24.0	33.0	31.25	13.6
14‡	0.2	2.5	3.5	3.3	2.2
15	1.35	15.5	23.0	21.65	11.7
16	0.34	1.6	2.0	1.66	1.3
17‡	0.135	3.0	4.8	4.665	9.0
18‡	0.34	0.405	0.54	0.20	0.15
19‡	0	0.135	0.135	0.135	<0.01
20‡	0	5.4	8.8	8.8	0.02
21‡	0.27	3.0	3.8	3.53	0.01
22‡	2.07	4.9	6.9	4.83	0.33
23‡	0.27	4.07	6.1	5.83	5.1

* Total individuals minus dead cells.
† Fat removal with heptane.
‡ Counted with 6× ocular.

zation does render a certain percentage of the killed bacteria unstainable when the standard direct microscopic method is used.

This preliminary work is sufficient to prove that the bacteria made invisible by pasteurization do not vanish, but that their stainability is often sufficiently reduced so that they do not show by methods requiring decolorization or when stains containing decolorizing agents are used. We did notice by our method, that bacteria killed by heat in aqueous suspensions

TABLE 10

A comparison of counts of Streptococcus lactis before and immediately after heating to 63°–65° C. for 30 minutes

Culture number	Before heating		After heating	
	Groups	Individuals	Groups	Individuals
1	1,200,000	5,250,000	1,200,000	4,050,000
2	1,600,000	5,400,000	1,070,000	4,200,000
3	1,350,000	2,800,000	1,200,000	3,200,000
4	1,900,000	4,050,000	1,750,000	4,700,000
5	810,000	3,500,000	675,000	1,500,000
6	400,000	1,900,000	810,000	2,300,000

TABLE 11

Effect of heating the milk to 63°–65° C. on microscopic counts made by Breed's method and by the cover-slip technique. Both counts are made on the same sample of milk

Sample number	Before heating		After heating	
	Groups	Individuals	Groups	Individuals
	Breed Smears			
1	2,800,000	7,000,000	1,700,000	4,100,000
2	1,800,000	6,200,000	1,800,000	3,500,000
3	13,000,000	42,000,000	9,200,000	20,500,000
4	5,400,000	7,000,000	4,300,000	5,200,000
5	1,050,000	3,600,000	600,000	1,250,000
	Cover-slip preparations			
1	4,450,000	12,200,000	5,000,000	11,600,000
2	2,100,000	4,750,000	2,100,000	3,800,000
3	17,000,000	31,600,000	19,500,000	24,700,000
4	5,100,000	18,700,000	6,250,000	16,000,000
5	2,200,000	4,750,000	2,000,000	5,050,000

are not as deeply stained as those killed by chemicals or by dry heat. It seems probable that this is due to the loss of the water-soluble, stainable materials from the cell, such as nucleic acid, lipoids, etc.

DISCUSSION

The soundness of the principles upon which the present method is based has been retested by us by observing the behavior toward our dye mixture, of viable cells and of cells killed by various means, including the quick cooling method of Sherman and Cameron (16). The preparations present an almost colorless background with the dead cells deep blue. The alkali accomplishes two purposes: In the first place, it makes the dye mixture sufficiently toxic to kill the viable cells; secondly, it acts as a mordant, intensifying the staining of these cells. This apparently dual rôle of the alkali is in reality brought about by the same mechanism, namely, the increased affinity between the cell substance and the dyes.

There is one objection to the method that has been already expressed to us, and that is the magnitude of the factor. The best we could do was to use 1.28×1.28 cm. cover glasses with the 6×97 combination. This gives us a factor of 1×10^6, which many people consider too large. We are not of this opinion. It is true that the high figures necessary in all direct microscopic methods constitute the limitation of these methods, yet we believe that the degree of contrast obtained in our technique, and the excellent distribution of the cells more than compensate for the relative increase in the factor. If 50 fields of a preparation be explored, a single cell encountered would be equivalent to 20,000 cells per cc. In many states this figure is not

too high for ordinary market milk. In any case, we are not suggesting our method as a substitute for the petri plate.

Other Possible Uses

This method may be used, not only in grading raw and pasteurized milk, with a clue to the history of the latter, but it can also be used in determining viable cells in butter cultures, and may also be modified for use in the bacteriological control of other dairy products.

SUMMARY

A direct microscopic method for distinguishing dead from viable bacterial cells in milk is reported. The method is based on the principles of vital staining. One cc. of the milk to be examined is mixed with 0.5 cc. of a dye mixture containing 0.1 per cent methylene blue and 0.1 per cent Nile blue sulfate, and with 0.5 cc. of a gelatin-agar mixture containing 8 per cent gelatin and 2 per cent agar. 0.01 cc. of this mixture is placed on a clean glass slide and covered with a small cover glass (1.28×1.28 cm.). The stained cells are then counted and they represent the dead cells already present in the milk. To a similar mixture one drop of 0.7 to 0.8 normal NaOH is added. This causes staining of all the cells present in the milk. The difference between the two counts represents the number of viable cells present.

The object of the agar-gelatin mixture is to solidify the preparation, keeping the bacteria in position. The factor is calculatd from a knowledge of the amount of milk present in the preparation and the area of the cover glass.

When desirable, fat can be removed by shaking the milk with heptane followed by moderate centrifuging.

A number of tables are included showing the results obtained when the method is applied to pure cultures and to pasteurized milk. The counts obtained are also compared to petri plate counts made on the same material.

The effect of pasteurization on the stainability of bacteria in milk is studied and briefly discussed.

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COD-LIVER OIL TOLERANCE IN CALVES

GEORGE K. DAVIS AND L. A. MAYNARD
Laboratory of Animal Nutrition, Cornell University, Ithaca, N. Y.

A previous extensive report from this laboratory by Madsen, McCay and Maynard (1) has described the production of muscle dystrophy in guinea pigs, rabbits, sheep and goats on rations containing cod-liver oil. While the injury occurred more regularly and with greater severity with synthetic diets containing the oil, it was clearly shown that rations of natural foods were made dystrophic by oil addition. The data indicated that the injurious factor resided primarily in the saponifiable fraction and no evidence was obtained that the vitamins present in the oil were in any way responsible for the lesions. These findings were extended in further studies with guinea pigs and rabbits by Madsen (2) at Columbia University, as a result of which it became evident that the severity of injury was influenced by certain dietary interrelationships.

During the past two years the problem has continued to receive attention in this laboratory, primarily in studies with guinea pigs, with the objects of identifying and eliminating the specific harmful factor and of learning more about the dietary interrelationships involved. These experiments are being reported elsewhere (3). During the same period, however, studies have been in progress with calves to ascertain whether this species is susceptible to the injury when cod-liver oil is used as a supplement to commonly fed rations. Such studies seemed particularly called for in view of brief reports by Slagsvold (4) and by Agduhr (5, 6, 7) of such an injury and in view of the increasing practice of using cod-liver oil or its products as vitamin supplements in calf feeding. Our experiments are described in the present paper.

The general procedure in these experiments has been to add graded levels of the oil to the rations of calves, beginning shortly after birth and continuing for periods ranging from six to nine months. The animals were then slaughtered for gross and microscopic examination of the organs and tissues. The same oil levels were used as had been employed in the previous studies with sheep and goats being reared on pasture, namely, 0.1, 0.35, and 0.7 gram per kilo live weight. The oil was the same product, designated as "animal grade," used in the previous studies, and stated to contain in excess of 1000 A. D. M. A. units of vitamin A and in excess of 150 A. D. M. A. units of vitamin D. Trials with guinea pigs had shown that a U. S. P. grade of oil was equally injurious. Two experiments were conducted with the calves. Since the rations employed and the procedures were somewhat different, the experiments are described separately.

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FIRST EXPERIMENT

Calves were purchased from nearby farms and brought to the University shortly after birth. After a brief period during which they were fed whole milk, given anti-scours serum and kept under veterinary observation, those which appeared disease-free and otherwise thrifty were placed on experiment. They were kept in individual pens. All of the animals were fed substantially alike with the exception of the cod-liver oil supplement. They received whole milk until three weeks of age, and then were gradually changed to skimmilk which was fed in accordance with their appetite with a maximum intake of 12 pounds per day. The feeding of skimmilk was discontinued at four and one-half months of age. The calves were given clover-timothy hay of good quality and the following grain mixture:

600 pounds wheat bran
500 pounds hominy feed
460 pounds ground oats
200 pounds linseed oil meal
200 pounds molasses
20 pounds bone meal
20 pounds salt

The calves were fed the hay *ad libitum*, and allowed all the grain they would eat up to a maximum of three pounds per head per day. The oil was given as a drench beginning at two weeks of age. Three animals received the highest level, two the intermediate level, three the lowest level, and two received 0.1 gram per kilo of a "reinforced" cod-liver oil[1] frequently used as a vitamin supplement for calves. Pertinent data regarding the calves are given in Table 1. The oil intakes were adjusted weekly in accordance with the weights of the animals. The amount of oil fed the calves receiving the highest level, however, was not increased beyond 200 grams per day, and in the case of the 0.35-gram level, not beyond 100 grams per day.

The animals were observed daily for any indications of unthriftiness or of more specific troubles. Weights were taken weekly. No digestive or other disturbances were noted on beginning the oil feeding. All of the animals grew well and remained in good condition during the first six weeks. Shortly thereafter, however, No. 9 started to lose weight and failed rapidly. The trouble was diagnosed as pneumonia and treatment was begun accordingly. The calf continued to fail and died after 62 days on the experiment. The post-mortem findings are discussed later.

All of the other animals continued to grow regularly and showed no evidence of unthriftiness. At 26 weeks one animal from each group was slaughtered for post-mortem examination and the remaining animals were

[1] This product was guaranteed to contain 400 U. S. P. units of vitamin D and 3000 U. S. P. units of vitamin A. It was prepared by adding to the straight oil a concentrate consisting of the non-saponifiable fraction.

TABLE 1

Records of calves used in first experiment

Calf	Breed	Sex	Supplement	Weeks on expt.	Av. daily gain	Muscle pathology
			gram per kilo		*lbs.*	
3	Holstein	♂	0.1 cod-liver oil	26	2.01	None
7	Holstein	♂	0.1 cod-liver oil	40	2.25	None
10	Guernsey	♂	0.1 cod-liver oil	40	1.78	None
4	Holstein	♀	0.35 cod-liver oil	40	2.34	None
8	Holstein	♂	0.35 cod-liver oil	26	2.25	Slight lesions
5	Holstein	♂	0.7 cod-liver oil	26	2.14	Very slight lesions
6	Holstein	♂	0.7 cod-liver oil	40	2.37	None
9	Guernsey	♀	0.7 cod-liver oil	7	.....	Slight lesions*
1	Holstein	♀	0.1 cod-liver oil (reinforced)	40	1.62	None
2	Holstein	♂	0.1 cod-liver oil (reinforced)	26	1.83	Slight lesions

* May have resulted from the pneumonia which caused the animal's death.

killed for the same purpose at nine months. The object here was to ascertain whether any injuries evident at six months tended to become more severe with the extension of the feeding period. The growth data presented in Table 1 indicate that all of the animals, with the exception of the one which died, made satisfactory gains for their breed and sex. In fact several made an excellent performance.

Clearly, aside from the case of the one animal which died, no evidence was obtained suggesting that any of the cod-liver levels used interfered with growth or condition. Of course, larger numbers and a check group would have been necessary to reveal any small differences in growth performance.

Post-mortem Examinations

It was recognized from studies with other herbivora that definite muscle lesions might develop without being sufficiently acute or widespread to cause physical evidence of injury over the periods studied. Thus each animal was subjected to a detailed gross post-mortem examination, and sections were taken from the leg, body and heart muscles, and from the liver, lungs, kidneys, adrenals and thyroid for histological examination. Several sections were taken from each muscle to insure discovery of isolated injuries. The general procedure was the same as previously described (1).

Of the three animals which received the 0.7-gram level of oil, the one which died after 62 days had revealed physical symptoms similar to those of the sheep and goats which had succumbed to the oil injury. The post-mortem examination confirmed the diagnosis of pneumonia, although the

amount of lung congestion seemed insufficient to have caused death. There were, however, no gross symptoms which suggested cod-liver oil injury. On histological examination slight lesions were found in the gastrocnemius muscle but these could not be considered proof of oil injury, in view of the presence of pneumonia which may cause similar lesions according to Forbus (8). Sections from other muscles failed to reveal evidence of dystrophy. The liver contained abundant fat, but it was not of a pathological nature. Thus the examination furnished no certain evidence that the cod-liver oil contributed to the death of this animal.

Calf No. 5 which was killed after receiving the 0.7-gram level of oil for six months appeared entirely normal on gross examination. Histologically, all the leg muscle sections examined showed slight pathological changes, indicative of dystrophy as seen in the other species. The remaining tissues and organs examined were normal. Calf No. 6 which was continued on the highest level of oil for nine months showed no pathological changes either in the gross or histologically.

Calf No. 8 which received the medium level of cod-liver oil was killed and examined at six months of age. The condition noted was almost identical to that observed in the calf on the high level killed at this time. The gross picture was entirely negative as regards pathological changes, but histologically some lesions were present in the museles of the legs. The injury was too slight to be of any real significance. The other calf on this level of oil, No. 4, was killed at nine months of age. It was normal in all respects according to both the gross and histological examination.

Of the animals which received the 0.1-gram level of oil, neither the one killed at six months nor the two killed at nine months revealed any gross or histological changes on post-mortem examination.

Calf No. 2 which was slaughtered after six months on the reinforced cod-liver oil showed no changes on gross examination, but histologically, isolated fibers of the leg muscles showed slight degeneration. The other animal fed this oil, killed at nine months, appeared entirely normal on post-mortem examination.

The results of this first experiment with calves are in marked contrast with those obtained with sheep and goats (1) where the same oil levels were fed. With these latter species severe lesions resulting in death were noted from the two higher levels whereas in the calves there was no physical evidence of injury, excluding the calf which developed pneumonia, and any histological changes were slight. They were not found at all in the animals continued for the longer period, suggesting the absence of the progressive injury which had been noted in the other herbivora studied.

SECOND EXPERIMENT

In view of the results obtained in the first experiment a repetition of the work to obtain data with more animals clearly seemed desirable. In

planning the second trial, it was decided to use a poorer grade of hay, of the kind that would warrant the inclusion of a supplement of vitamins A and D in practice. Accordingly, a timothy hay which was rather stemmy and lacking in color was selected. The choice of this low-protein roughage necessitated a concentrate mixture of higher protein content than the one used in the first experiment. It was made up as follows:

520 pounds gluten feed
100 pounds cottonseed meal
200 pounds wheat bran
60 pounds hominy or corn meal
220 pounds brewers' dried grains
140 pounds corn distillers' dried grains
100 pounds soya bean oil meal
400 pounds oat feed
220 pounds molasses
20 pounds bone meal
20 pounds salt

A further reason for using this ration was the findings of Turner, Meigs and Converse (9) that with the same intake of cod-liver oil rabbits developed the injury much more quickly and severely on a poor than when on a good ration. With the object of keeping the fat intake low, in view of evidence obtained by Madsen (2) as well as by ourselves that certain fats tended to lessen the injury in guinea pigs, the calves were changed to skimmilk during the first week after birth. Thus the basal ration was chosen with the object of providing the conditions where the cod-liver oil injury would be most likely to develop, based on observations with other species, as well as of providing a ration which in practice would call for the addition of the vitamins supplied by the oil.

Five grade Holstein calves were purchased and brought to the University within three days after birth. Following treatment with anti-scours serum and a few days observation they were placed on experiment in a common pen providing for the individual feeding of the milk. The intake of the latter was progressively increased up to a maximum of 15 pounds per day. At four and one-half months the milk feeding was discontinued. The grain and hay were fed *ad libitum* throughout the experiment. Three of the calves received the 0.7-gram level of the oil and two received the "reinforced" oil at the 0.1-gram level, as shown in Table 2.

During the first four weeks all of the calves grew regularly and appeared thrifty. The growth was somewhat below normal, however, which could be explained as being due to the early change to skimmilk. During the fifth week calf No. 968, receiving the high level of oil, became stiff and showed symptoms of pneumonia. It was killed in a morbid condition ten

TABLE 2

Records of calves used in second experiment

Calf	Sex	Supplement	Weeks on expt.	Av. daily gain	Muscle pathology
		gram per kilo		*lbs.*	
966	♂	0.7 cod-liver oil	24	1.84	Lesion in one fiber
967	♀	0.7 cod-liver oil	24	1.16	Slight lesions
968	♂	0.7 cod-liver oil	6	.....	None
964	♂	0.1 cod-liver oil (reinforced)	24	1.80	None
965	♂	0.1 cod-liver oil (reinforced)	24	2.27	None

days later, having lost the use of its hind legs. At five weeks another calf, No. 967, developed pneumonia. By careful treatment the trouble was cleared up, although the animal continued to cough until it was three months of age. For a month the calf made little growth, but after three months, growth occurred at a normal rate until it was killed for post-mortem examination at six months of age.

The other three calves remained in good condition throughout the experiment and grew regularly. As is shown in Table 2, the average daily gains for the period as a whole were very good despite a poor growth during the first four weeks. The low average daily gain for No. 967 reflects its set-back caused by pneumonia. At 24 weeks the calves were killed for post-mortem study.

Post-mortem Examinations

Calf No. 968 which became stiff and died after six weeks revealed a slight pneumonia on post-mortem examination, but not sufficient to cause death. The most marked finding was the presence of a very large, dry mass of hay in the rumen. The physical symptoms before death were very similar to those shown by sheep and goats in which severe muscle lesions were found. Histological examination of this calf failed to show any changes from the normal in the muscles, liver, kidneys or gastrointestinal tract. There was no evidence that the oil was in any way responsible for the failure of the animal.

Calf No. 967 which recovered from pneumonia and grew normally during the last three months showed no pathological changes on gross examination. Histologically, however, both the leg and body muscles showed definite lesions. While these lesions were slight, they were widespread and characteristic of cod-liver oil injury. Since careful study of the lungs showed that the calf had completely recovered from the pneumonia, any muscle lesions caused by this disease should have been healed. Thus it is believed that the lesions found were caused by the oil. Histological studies of the heart, kidneys, liver and thyroids revealed no pathological changes.

The remaining animal receiving the high level of oil was in excellent condition when killed and showed no gross changes on post-mortem. Of the sections taken from the various muscles for histological examination, only one showed any change from normal, and the degeneration was present in only a single fiber. Such an isolated injury is of no significance.

None of the calves which received the "reinforced" oil revealed any evidence of pathological change either in the gross or histologically.

DISCUSSION

The results of these two experiments clearly indicate that dairy calves are much less susceptible to cod-liver oil injury than are sheep and goats. With the latter species, according to the previous studies (1) an intake of 0.7 gram per kilo resulted in death or a morbid condition within 90 days, and the 0.35-gram level produced similar results after a longer period. The post-mortem examinations revealed widespread and severe lesions. In contrast, the calves fed these levels showed no physical symptoms of injury over periods of six and nine months. On post-mortem examination most of them were found free from either gross or microscopic lesions and in the others pathological findings were limited to slight microscopic lesions.

The experiments with the different species are not strictly comparable because the rations to which the oil was added were very different. This fact needs emphasis in view of the data previously mentioned indicating that dietary interrelationships have an influence on the degree of injury, but it is not believed that this can be the major explanation of the very different results obtained with the calves than with the other herbivora.

Agduhr and Stenstrom (10) reported that they detected alterations in the heart muscle of calves fed cod-liver oil, by means of the electrocardiogram. In view of this report electrocardiograms were taken at regular intervals on the calves included in the present study. No alterations could be detected as a result of the cod-liver oil feeding. Thus both these studies, which are described elsewhere by Barnes, Davis and McCay (11) and the histological studies here reported failed to confirm the observations of the previous workers as to heart damage in calves from feeding the oil. Here again possible differences due to the nature of the basal rations should be kept in mind.

While the striking feature of the present results is the slight nature of any changes noted, contrary to our previous results with other herbivora and to the results reported by the Swedish workers with calves, our observations do indicate that cod-liver oil can cause muscle injury in calves. At least three of the animals receiving the two higher levels revealed definite, though slight lesions which could not be attributed to any other cause, and one animal receiving the "reinforced" oil showed injury to a similar degree. It is probable that the lesions noted were too slight to interfere with

the normal development of the animals unless further feeding of the oil would have intensified them. Since all the cases of lesions were noted in the animals killed at six months, the animals killed at nine months showing no abnormalities, no evidence was obtained for a progressive injury such as we have frequently noted with the other herbivora studied. Perhaps more marked lesions would have been found if the animals had been examined earlier than at six months.

In the same paper in which their studies with rabbits were described, the Beltsville workers (9) reported observations with calves fed cod-liver oil. An intake of 0.7 gram per kilo caused no evident physical injury, but the high level of two grams per kilo resulted in death. By arrangement with Doctor Meigs tissue sections from some of these calves and from those on later experiments carried out at Beltsville have been studied histologically by us. The findings[2] will be reported in detail from Doctor Meigs' Laboratory. It may be stated, however, that muscle degeneration has been found in ten out of 13 calves studied, ranging from a slight to a moderately severe degree, supporting the observations in our own experiments that cod-liver oil can cause muscle dystrophy in calves. In most cases the intakes fed by the Beltsville workers were higher than those used by us, explaining the greater incidence of injury in their animals.

The observation of cod-liver oil injury in calves has a bearing on feeding practice in that the oil finds some use as a supplement to calf rations to supply vitamins A and D. Long and associates (12) have reported that calves require .3 to .4 U. S. P. units of vitamin D per pound of body weight in addition to the amount supplied by whole milk. Bechdel and associates (13) have reported the much higher requirement of 300 U. S. P. units per 100 pounds live weight where skimmilk was used after four weeks. This intake would be supplied by a U. S. P. grade of cod-liver oil fed at a level of approximately .08 gram per kilo.

Since no sign of injury was noted in the present studies where 0.1 gram per kilo was fed, and since the lesions were slight at higher levels, it appears that the amount of cod-liver oil of U. S. P. grade or higher potency required to furnish the vitamin D needs of the calf can be added to the commonly fed rations used in the present studies without fear of significant harm. Since a much lower intake of a fortified oil would be required, there would seem to be no question about its being a safe source of the vitamins.

The present experiments provide no information as to the conditions under which a supplement of vitamin A or D is needed in a calf ration, and thus the preceding statements should not be interpreted as recommending the general use of cod-liver oil or any other supplement of the vitamins for calves. Evidence such as that obtained by Dahlberg and Maynard (14) indicates that with proper roughage no such supplement is needed.

[2] The writers appreciate the courtesy of Doctor Meigs in allowing them to cite these unpublished results.

SUMMARY

Dairy calves were reared from birth to six or nine months of age on skimmilk, hay, and grain, supplemented with cod-liver oil at different levels, ranging up to 0.7 gram per kilo. No evidence was obtained that the oil affected adversely the growth or physical condition. On killing the animals for post-mortem study, no gross changes indicative of cod-liver oil injury were found. On histological examination of various muscles, slight dystrophic changes were found in some of the animals, primarily in those fed the higher levels; but the lesions were of a very minor character, in marked contrast to previous findings with other herbivora. The results suggest that cod-liver oil can be fed to dairy calves in the amount that may be required to supply vitamin D without any significant injury.

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HAY CONSUMPTION OF HOLSTEIN CALVES

H. S. WILLARD
Wyoming Agricultural Experiment Station, Laramie, Wyoming[1]

Factors which may influence hay consumption of dairy calves are of interest to many farmers who have a limited amount of grain to feed and who wish to take advantage of the economical growth made by calves when compared with older stock. It is generally recognized that calves do not have a large capacity for feed and that the need of energy for desirable growth necessitates the feeding of more concentrated feeds than hay alone. The questions arise as to what age or weight a calf may consume sufficient roughage to give it the desired energy on hay alone, and what are the factors influencing greater hay consumption. A review of the literature on hay consumption by calves reveals a wide variation in the hay reported as consumed and gives little assistance in answering the questions.

This article reviews some of the work conducted at the Wyoming Agricultural Experiment Station as an attempt to clarify the subject.

METHOD OF PROCEDURE

The standard for rate of growth was taken from an average of experiment station data as published by Morrison (4). The nutrient intake used as a guide in formulating rations was the Morrison standard as modified by Fitch and Lush (2). The rations fed were designed for farms where whole milk is sold. In formulating rations to conform with the Morrison standard, average analyses of feeds as found in Morrison's "Feeds and Feeding," nineteenth edition, were used.

One group of eight registered Holstein heifer calves, hereafter called Group 1, were fed according to the Morrison feeding standard in individual box stalls from birth to twelve months of age. Whole milk was fed until the calves were about three months of age. For the first three months the grain allowance was unlimited; thereafter, it depended upon the consumption of hay. However, the grain allowance never got below two pounds per head daily. The calves were never allowed to go on pasture, but had access to a dry lot for about five hours during the day. The hay and grain were weighed out to the calves daily, and all refused feeds weighed back. Daily weights of all calves were taken. When the calves were twelve months of age, they were measured for height at the withers, and heart girth.

The grain mixture consisted of three parts ground barley, two parts ground oats, and one part wheat bran. One per cent common salt was added

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[1] Published with the permission of the Director of the Wyoming Agricultural Experiment Station.

to this mixture. Uncut alfalfa hay was fed as a sole roughage. For the most part the alfalfa was of high quality, ranging between 16 per cent and 18 per cent in crude protein. Oat and wheat straw was used for bedding. The calves had access to water in the dry lot and to drinking cups in the barn, except for short periods when the water in the barn had to be shut off to prevent freezing in the pipes.

Another group of eight registered Holstein heifer calves, hereafter known as Group 2, were fed in a manner similar to those in Group 1 until they were about eight months of age. At that time the grain allowance was gradually decreased and the hay allowance increased until the calves were on hay alone at an average of ten months.

RESULTS

The average hay and grain consumption by weight for Groups 1 and 2 is plotted in Figure 1, which shows that in a single group, when the grain fed

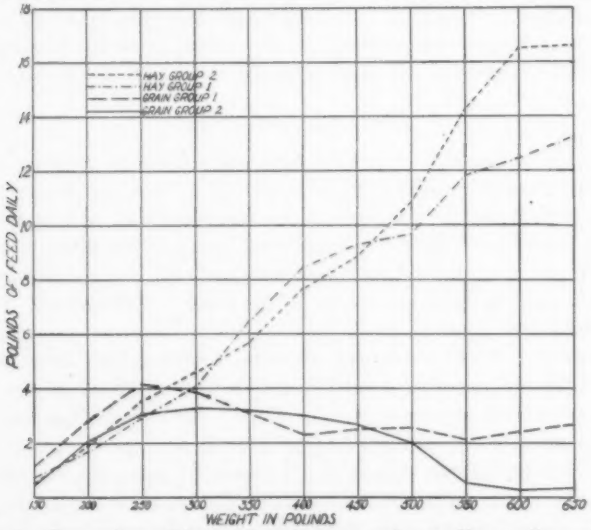

FIG. 1. Comparison of hay and grain consumed according to live weight.

was high, the hay consumption was low. This situation is not necessarily caused by the relative palatability of the hay, although the quality of the hay might have been a factor. When calves in Group 2 had reached about 500 pounds in weight, the hay allowance was increased and the grain decreased, until it was found that the calves were consuming close to the Morrison requirements, using hay alone. The calves were 300 days of age on an average when the grain feeding was discontinued. There are two known factors which made possible this higher consumption of hay: (1) increased allowance of hay, and (2) decreased allowance of grain.

With the grain and hay consumption going in opposite directions it might be anticipated that the calculated total digestible nutrients consumed by the groups would be similar. That such was the case is shown in Figure 2 where

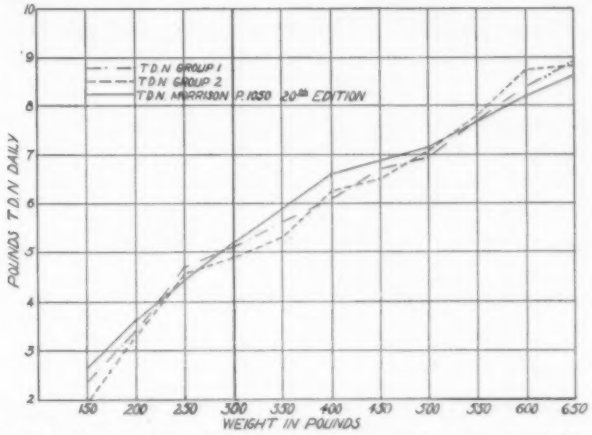

FIG. 2. Calculated and digestible nutrients consumed by Wyoming heifers compared with Morrison's standard.

the T.D.N. consumed by weight is plotted and compared with the average of the Morrison standard.

Increased allowance of hay is a factor influencing hay consumption. Figure 3 shows that the hay consumption of the two groups of calves is related very definitely to hay allowance. It would appear from Figure 3 that Group 1 refused the greater amount of hay. On an average Group 1 refused 12 per cent and Group 2, 7.5 per cent of the hay offered. The greater hay consumption at certain periods of growth in both Groups 1 and 2 was not due to a greater hay allowance alone, but to the amount of grain fed as well, because whenever an increase in the amount of hay fed was made, there was a corresponding decrease in the allowance of grain. To separate the influence of either one of the two factors involves some complications. Considering the feed allowed and consumed between the ages of 180 to 360 days, the calves of Group 1, on an average, were allowed 78 pounds less hay, 143 pounds more grain, and consumed 207 pounds less hay than the calves in Group 2. There was, therefore, a greater difference in consumption between the two groups than there was in hay allowance, indicating that the greater amount of grain fed to calves of Group 1 was a factor depressing the hay consumption. In Figure 4 the grain consumption of each individual calf is plotted from high to low amounts, together with the corresponding hay allowance and consumption, indicating a trend toward a greated consumption of hay with a decreasing grain allowance. However, there are notable exceptions to this

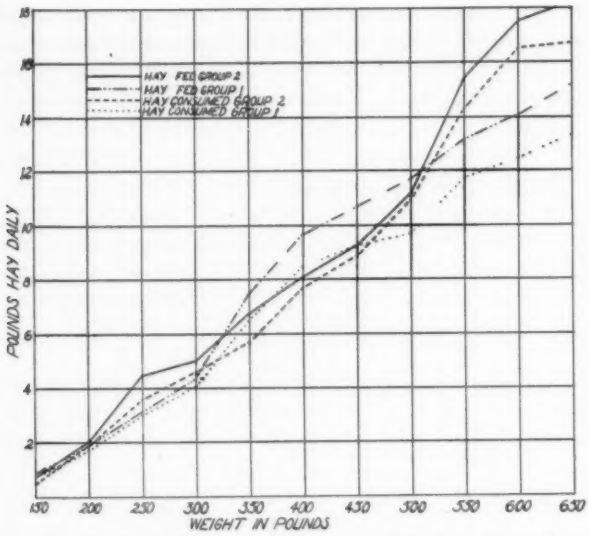

FIG. 3. Comparison of hay allowance with hay consumed.

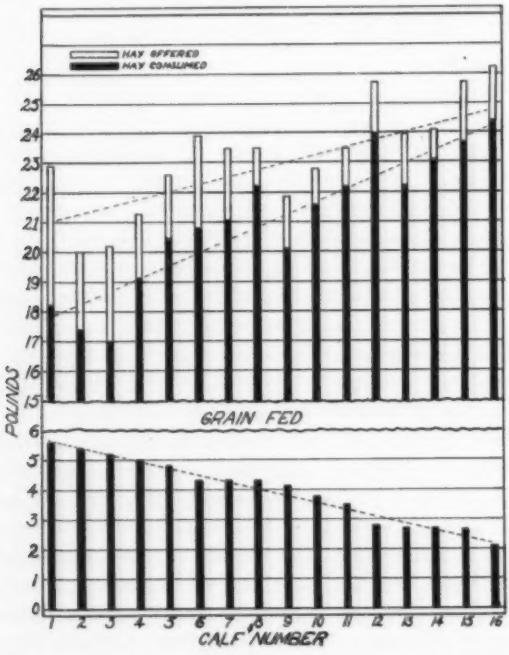

FIG. 4. Effect of grain allowance on hay consumption of 16 calves from 180 to 360 days of age.

trend. Calves 6, 7, and 8 consumed similar amounts of grain, but their hay consumption, indicating a trend toward a greater consumption of hay with Calves 2 and 3, the unusual decline in consumption was due to the smaller hay allowance.

The data in Figure 4 can be used only to indicate trends, since, throughout the experiment, there was a marked difference in the appetites for hay of individual calves.

The rate of growth of the two groups of heifers is compared, in Figure 5,

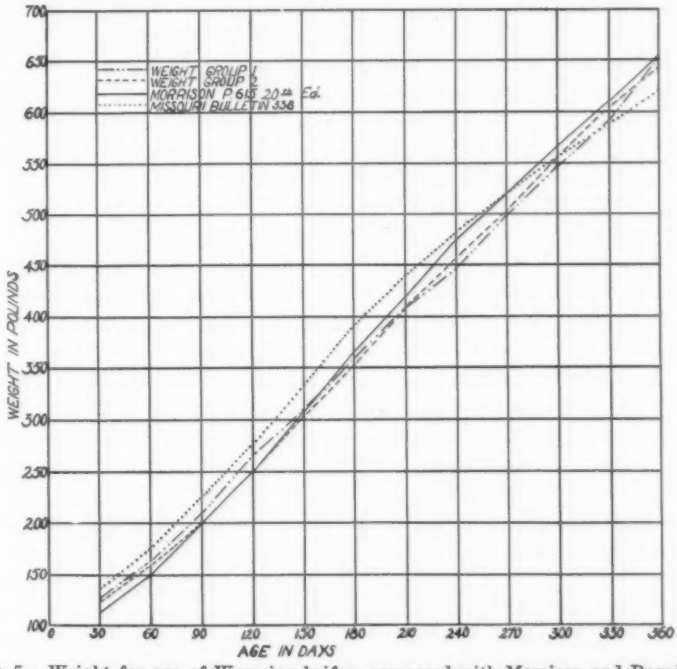

FIG. 5. Weight for age of Wyoming heifers compared with Morrison and Ragsdale.

to that published by Morrison based upon records from several experiment stations, for Holstein heifers (4), and the data by Ragsdale (5). There is, as shown in Figure 5, similar growth curves indicating that the rate of growth of the two Wyoming groups compares favorably with heifers raised where the animals secure greater abundance of feed than they receive on many farms.

COMPARISON WITH OTHER DATA

There is little need for comparing the hay consumption of the two groups of Wyoming heifers with that reported in the literature of calf rations, other

than to emphasize possible changes in recommendations in hay allowances when calves are fed on a maximum of high quality alfalfa hay. It was difficult to secure figures comparable with the Wyoming data, because much of the published material gives the total consumption at a given weight or age. In much of the data, skimmilk was fed up to six months of age, and silage was fed as part of the roughage from the ages of six to twelve months. For the most part, when consumption of hay is given by weight or age it is recorded as the total or average daily consumption by months. Comparisons with such data, while not exact, at least show trends which are of value.

In Figure 6 the hay consumption of the Wyoming heifers is contrasted

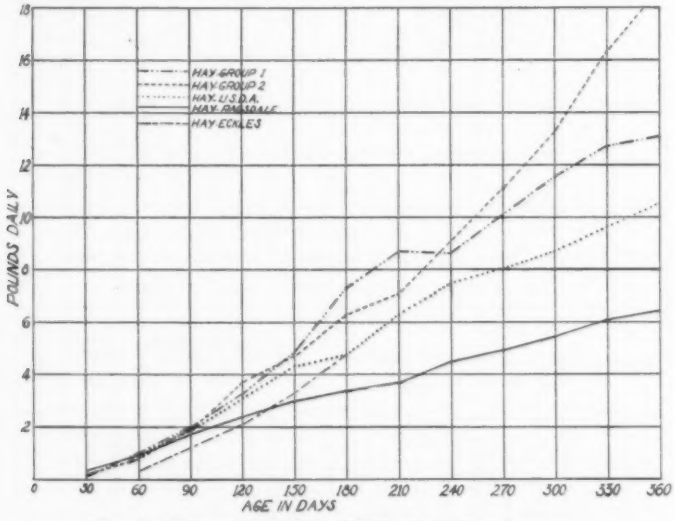

FIG. 6. Hay consumption of Holstein heifers by ages.

with data from three other sources. Eckles (1) reported average daily hay consumption by ten day intervals for Holstein calves from birth to six months of age, fed on rations designed for farms where whole milk is sold. Ragsdale (5) reported the average daily feed consumed by months by Holstein heifers at the Missouri Agricultural Experiment Station, feeding corn silage as part of the roughage. The Shepherd and Miller (6) report on feed consumption also shows that corn silage was fed. In order to compare the roughage consumption in terms of hay, the silage consumption has been divided by 3 and added to the hay consumption, and the total plotted in Figure 6. It is apparent that the roughage consumption reported by Shepherd and Miller comes closest to the Wyoming data, but the spread becomes greater beginning with the eighth month. During the eighth month of age, the average daily hay consumption for Wyoming Group 2 was 12 per cent higher than that

reported by Shepherd and Miller, and the spread continues to widen with increasing age of the heifers. During the twelfth month of age, the Wyoming Group 2 heifers consumed 74 per cent more hay.

DISCUSSION

When feeding calves according to a certain total digestible nutrient intake by weight, on rations containing a high percentage of hay, it is difficult to ascertain whether the maximum hay consumption has been reached without a decline in the total digestible nutrient intake of the entire ration. The high hay consumption and similar total digestible nutrient intake was secured in the Wyoming Group 2 heifers simply by trial, lasting sometimes a week or ten days. If, by increasing the hay allowance and decreasing the grain allowance, the heifers failed to consume the required total digestible nutrient intake, then the grain allowance was raised.

Experiments are now under way at the Wyoming Experiment Station to use this method as a means of testing the relative palatability of hay. Exactness in following a schedule for total digestible nutrient intake cannot be expected. In the first place, in experiments of this kind, the exact total digestible nutrient intake would be difficult to secure without a digestion trial. In the second place, heifers vary in weight from day to day, and also vary daily in their hay consumption. Gain in weight and high feed consumption coincide only as a trend and not from day to day.

Using average total digestible nutrient values was satisfactory in these instances, because the object sought was not to find the exact requirements for growth, but rather to find some of the factors which produced great hay consumption so that cheaper growth might be made possible. Net energy values for hay and concentrates may have been more accurate measures here because it is generally recognized that the net energy values of hay and concentrates are farther apart than the total digestible nutrient values would lead one to believe. With all of these possible sources of error in estimating nutrient values, it is interesting to note that the rate of growth of the two groups of calves was practically identical.

SUMMARY AND CONCLUSIONS

In this study, two factors have been demonstrated as having an effect toward increasing the consumption of alfalfa hay by dairy calves: (1) an increase of hay allowance, (2) a decrease of grain allowance. This study has further shown that calves may make desirable growth when fed high quality alfalfa hay alone, as early as nine months of age. The experiments also furnish a basis for further study of factors influencing hay consumption.

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THE USE OF YEAST IN CALF MEALS AND PELLETS[1]

PAUL E. NEWMAN AND E. S. SAVAGE
Cornell University, Ithaca, N. Y.

There has long been a need on dairy farms selling whole milk for a satisfactory method of growing calves with the use of only a small amount of milk.

Remixed dried skimmilk has been used successfully. The increased use of dried skimmilk in human foods has often brought the price of this product up to a point which makes the remixed dried skimmilk method expensive. The labor and care required in remixing the dried milk, heating water, and cleaning pails are also serious disadvantages of this method.

Various gruels have been used. The gruel method, however, is generally unsatisfactory, both from the standpoint of the development of the calves and the labor and care required.

During the past several years progress has been made in raising calves on a small amount of whole milk and various dry calf meals.

A number of studies have been made in which whole milk feeding has been limited to 60 days or less and a simple mixture limited largely to home grains, wheat bran, and linseed meal used (1, 2, 3, 4, 5). The growths of the calves in these studies were quite generally below normal.

Other studies have used dry calf meals containing a wide variety of ingredients and substantial amounts of either dried skimmilk or blood flour or both (5, 6, 7, 8, 9, 10, 11, 12). The results on these various dry calf meals have been somewhat variable. Some have produced very satisfactory growths in calves from the time the calves were weaned from whole milk at from six to ten weeks of age. Others have resulted in growths below normal while the calves were young but by the time the calves were six months of age they were usually of normal size or above.

Previous work at Cornell University (13) on this problem led to the recommendation of a formula for a dry calf starter, and a method of raising calves by the use of this formula.

The purpose of the experimental studies here reported was to test the ability of the recommended method to grow satisfactory calves consistently and to study changes which might further improve it. The latter phase of the work was concerned principaly with the use of yeast in the formula and the effects of pelleting the calf starters.

[1] This work was made possible through the financial assistance of Anheuser-Busch, Inc., St. Louis, Missouri. The material included was also presented to the Faculty of the Graduate School of Cornell University, June, 1937, by Paul E. Newman in partial fulfillment of the requirements for the degree of doctor of philosophy.

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Definite information regarding the value of yeast for calves receiving a minimum of whole milk is very limited. Eckles and associates (14) found that the addition of either a dried baker's yeast or a live yeast to the ration of calves receiving whole milk or a combination of whole milk and skimmilk had no effect on the growth or health of the calves. Yeast is known to be high in the vitamin B complex particularly vitamin G (15). Earlier work (16) indicated that what was then (1926) considered vitamin B was not needed in the rations of calves. However, this work was largely with calves after the milk feeding age. In addition the conception of the vitamin B complex has changed greatly since that time.

EXPERIMENTAL PROCEDURE

In the studies 65 calves were fed by the dry calf starter method as previously recommended by Cornell University (13). Most of the calves were Holsteins with a few Ayrshires and Guernseys.

The dry calf starter method which was used included the following essential features: Each calf was fed 350 pounds of whole milk. The whole milk fed per day was distributed over the first seven to ten weeks as follows:

POUNDS WHOLE MILK FED DAILY

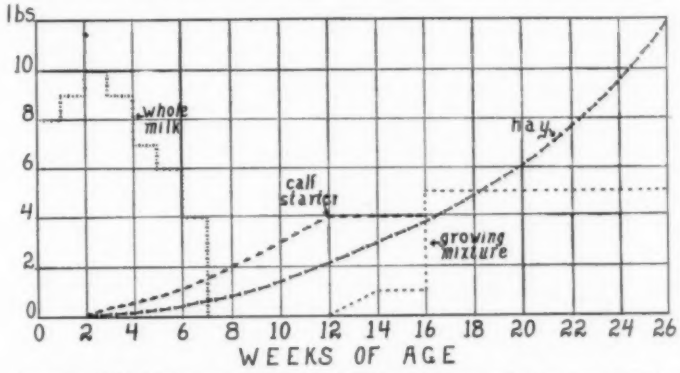
	Holstein	*Ayrshire*	*Guernsey*
Calf left with cow first day			
2nd to 7th day, not to exceed	8	7	5
2nd week, not to exceed	9	8	6
3rd " " " "	10	9	7
4th " " " "	9	8	7
5th " " " "	7	7	6
6th " " " "	6	6	6
7th " " " "	4	4	5
8th " " " "	0	3	4
9th " " " "	0	0	3
10th " " " "	0	0	3

Total milk fed was 350 pounds in each case.

An experimental dry calf starter was fed the calves *ad libitum* from two weeks of age until the average daily consumption reached four pounds per calf. It was held at this level until the calf starter was discontinued at sixteen weeks.

After a four pound level of the starter had been reached an additional one pound of a growing mixture was allowed for the remainder of the experimental period in addition to the calf starter. The growing mixture was a 13 per cent protein mixture consisting on a percentage basis of wheat bran 25, corn 25, barley 14, oats 10, linseed meal 10, cane molasses 14, bone meal 1, and salt 1.

Clean fine-stemmed leafy mixed hay was fed the calves *ad libitum* in hay racks beginning at two weeks of age. It was necessary to use three different lots of hay. One was classed as clover medium timothy mixed hay, the second as alfalfa heavy grass mixed hay, and the third as alfalfa heavy timothy mixed hay. A small amount of the second lot graded U. S. No. 1, the balance of the hay used graded U. S. No. 2.



Average daily feed consumption of Holstein calves used in the experiments.

With the exception of the differences in the calf starter formulas which were being studied, all calves were fed and handled as nearly alike as possible. Clean water was available to the calves at all times. Milk was fed the calves individually. The other feeds were fed by groups of two to four experimental calves of approximately the same age.

The experimental period ended at sixteen weeks of age.

The ingredients of the principal experimental calf starter formulas studied are given in Table 1. Formula A, the check formula, is the one previously recommended (13).

The cereal yeast feed used was produced by combining a yeast (Anheuser-Busch strain "L") with corn gluten feed and corn germ meal. This product was approximately 60 per cent corn gluten feed, 20 per cent corn germ meal, and 20 per cent yeast on an 8 per cent moisture basis. It contained 8.0 per cent water, 28.0–29.0 per cent protein, 4.0–4.5 per cent ether extract, 6.0–7.0 per cent fiber, 48–50 per cent nitrogen free extract, and 4.0 per cent ash.

RESULTS AND DISCUSSION

Examination of Table 2 shows that on each of the starters the average growth during the experimental period, 2 to 16 weeks of age, was above Ragsdale's normal (17).

TABLE 1

Ingredients of experimental calf starters

Formula	A	AY	GY	GYS	S	3M
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Ground yellow corn	32.25	29.00	26.25	32.25	33.25	26.25
Rolled oats (oat meal)	28.00	25.00	28.00	28.00	28.00	24.00
Wheat bran	10.00	10.00	10.00	10.00	10.00	10.00
Linseed meal	5.00	5.00	5.00	5.00	5.00	5.00
White fish meal	3.00	3.00	3.00	3.00	3.00	3.00
Dried skimmilk	*20.00*	*20.00*	*10.00*	*10.00*	*10.00*	*30.00*
Dried brewers' yeast	.....	*6.25*	.....	.....	.....	.....
Cereal yeast feed	.....	.....	*16.00*	*5.00*	.....	.....
Soybean oil meal	.....	.....	.....	*5.00*	*9.00*	.....
Steamed bone meal	.50	.50	.50	.50	.50	.50
Ground limestone	.50	.50	.50	.50	.50	.50
Salt	.50	.50	.50	.50	.50	.50
Cod-liver oil concentrate	.25	.25	.25	.25	.25	.25
Per cent total protein	20.2	22.4	19.4	19.2	20.5	22.1

The average growth of 14 calves on the check formula (A) was 112.8 per cent of normal. For formula AY which had 6.25 per cent dried brewers' yeast added to formula A the average growth of 13 calves was 133.7 per cent of normal. The 9 calves on formula GY in which the dried skimmilk of formula A was reduced to 10 per cent and 16 per cent cereal yeast feed was used averaged 142.3 per cent of normal in growth. In formula GYS the dried skimmilk was reduced to 10 per cent and 5 per cent each of cereal yeast feed and soybean oil meal used. On formula GYS the average growth of 18 calves was 132.9 per cent of normal.

It appears to be quite significant that the growth of the calves on each of the three formulas containing yeast was definitely greater than the growth of the calves on the check formula and approximately one-third greater than what is considered to be normal growth. The numbers are large, 40 calves on the yeast containing rations and 14 on check ration A.

The general appearance, condition, and body development of the calves on the three yeast formulas was fully as good, and perhaps somewhat better. On all four of the formulas, however, the calves were especially pleasing in these respects.

Formula S in which the dried skimmilk was reduced to 10 per cent and 9 per cent of soybean oil meal but no yeast was used, gave practically as large growths as the check formula (A). The calves were less uniform, however, in growth, appearance, and condition.

The use of 30 per cent dried skimmilk in formula 3M resulted in slower growth than on the check formula. The reason appeared to lie in the lower palatability of the 30 per cent dried skimmilk formula. The bloom and appearance of these calves was very pleasing at all times.

TABLE 2

Summary of growths of calves on various formulas

Formula	Total number of calves	Per cent normal weight		Average daily gain from 2 to 16 weeks, pounds			Per cent normal ave. daily gain 2 to 16 weeks, all breeds	Per cent 16 weeks weight is of 2 weeks weight
		2 weeks	16 weeks	Holstein	Ayrshire	Guernsey		
A	14	91.6	103.4	(11 calves) 1.44	(2 calves) 1.30	(1 calf) .97	112.8 ± 4.0	256.7 ± 5.4
AY	13	98.4	117.9	(10 calves) 1.70	(1 calf) 1.33	(2 calves) 1.34	133.7 ± 5.7	272.4 ± 5.8
GY	9	102.9	123.1	(5 calves) 1.77	(2 calves) 1.66	(2 calves) 1.28	142.3 ± 5.7	282.5 ± 3.4
GYS	18	96.5	116.7	(14 calves) 1.71	(3 calves) 1.60	(1 calf) .85	132.9 ± 4.5	275.4 ± 5.0
S	6	98.9	109.3	(6 calves) 1.52	.....	.....	117.7 ± 7.1	251.3 ± 6.4
3M	5	100.6	101.6	(5 calves) 1.32	.....	.....	102.5 ± 5.9	231.4 ± 13.1

NOTE: In the last two columns of the table are given the means and their standard errors computed according to the following formula:

$$\sqrt{\frac{\Sigma(X^2)n-(\Sigma X)^2}{n^2\ (n-1)}}$$

The normals used are those of Ragsdale (17).

TABLE 3

Effects of yeast on feed consumption and efficiency of gains
(Only lots consisting entirely of Holsteins are included.)

Formula	A	AY	GY	GYS	S
Number of calves	8	7	4	8	6
Total digestible nutrients consumed per calf, 2 to 16 weeks, pounds	330.6	340.8	360.3	350.3	378.4
Total gain per calf, pounds	138.6	156.6	164.2	157.2	148.9
T.D.N. per 100 pounds gain	238.5	217.6	219.4	222.8	254.1

In Table 3 the relative feed consumption and efficiency of gains on the yeast formulas compared to the check formula and the soybean oil meal formula are shown. The table is only for lots made up entirely of Holsteins as lots of more than one breed could not be included.

Apparently the variation between lots in the consumption of total digestible nutrients was not related to the yeast contents of the formulas. Observations during the experiments indicated that yeast neither increased nor decreased the palatability of the formulas. These figures indicate that in addition to having no influence on palatability yeast had no definite effect on increasing the appetite or total food consumption as is sometimes claimed for it.

The figures on total digestible nutrients required per 100 pounds gain show that the gains were made more efficiently in terms of nutrient utilization on the yeast formulas than on the non-yeast containing formulas.

Half of the calves fed formulas A and AY were fed these formulas in the meal form. The others were fed the same formulas in the pellet form. The pelleting was done by a commercial company equipped to make calf pellets. The pellets were rather hard and approximately three-sixteenths of an inch in diameter.

Table 4 shows that there was no increase in growth, in fact, the growth was somewhat less, on the pellet form compared with the meal form of those two starters. The reason for this is probably due to a lower consumption of the pellets compared with the meal while the calves were young (two to six weeks). This was true for each formula.

TABLE 4

Average growth of calves fed the meal and pellet forms of formulas A and AY

Number of calves	Meal	Pellets
	14	13
Average daily gain 2 to 16 weeks, % normal	127.1 ± 4.9	118.3 ± 6.1
% 16 weeks weight is of 2 weeks weight	270.8 ± 6.0	257.2 ± 5.3

SUMMARY AND CONCLUSIONS

Sixty-five calves were used in studying different calf starter formulas. The following conclusions seem to be warranted:

1. The dry calf starter method in these studies consistently produced calves above normal in weight and of desirable body and skeletal development.

2. The use of dried brewers' yeast and cereal yeast feed in the dry calf starter formulas resulted in greater growth and body development.

3. The total digestible nutrient requirement per unit gain in weight was lower for the yeast-containing calf starters than for the others.

4. The previous level of 20 per cent dried skimmilk can be reduced to 10 per cent without decreasing the effectiveness of the calf starter when yeast or a combination of yeast and soybean oil meal is used to keep the protein level the same.

5. Using a level of 30 per cent dried skimmilk lowered the palatability of the starter and resulted in slower growth.

5. Pelleting of the two formulas studied decreased the consumption of the calf starter by young calves and resulted in slightly slower growth.

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American Dairy Science Association Announcements

THE THIRTY-THIRD ANNUAL MEETING

The thirty-third annual meeting of the American Dairy Science Association will be held at The Ohio State University, Columbus, Ohio, and The Ohio Agricultural Experiment Station, Wooster, Ohio, June 14 to 17, 1938. The concluding sessions on June 17 will be held at Wooster and an interesting general program is being arranged. We are inviting speakers for this part of the program whom everyone in the industry will be interested in hearing.

LAST CALL FOR TITLES AND ABSTRACTS

The cooperation of each speaker in the prompt submission of titles and abstracts has, in the past, made it possible to have the abstracts published and ready for distribution at the time of the meetings. This year the plan is to have the abstracts of the papers to be presented published and in the hands of the membership before they leave for the meetings. This necessitates the advancement of the deadline for receiving titles and abstracts to a date somewhat earlier than in the past.

April 15 is the deadline, and the program committee will appreciate your cooperation by having your abstract and title at a somewhat earlier date.

Send titles and abstracts to T. S. Sutton, Department of Animal Husbandry, Ohio State University, Columbus, Ohio.

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SPECIAL PUBLICATIONS

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ABSTRACTS OF LITERATURE

BACTERIOLOGY

73. A New Microscope with Photographic Chamber. A. KARSTEN. Le Lait *17*, 164, p. 337, April, 1937.

A microscope is described in which one can simultaneously observe the object and photograph it. Application of the instrument to photographing bacterial slides and plates and filtration fibers is discussed. A.H.J.

74. Dyes and Methods as They Affect the Methylene Blue Test. J. M. FRAYER, Univ. of Vermont, Burlington, Vt. Vt. Agr. Exp. Sta. Bul. 424, June, 1937.

Three methods of conducting the methylene blue reduction test were compared, using 50 to 100 replicate tests of each sample being made.

1. Substantially as directed in the sixth edition of "Standard Methods of Milk Analysis."
2. Essentially, according to proposed changes to be embodied in the seventh edition of "Standard Methods for the Examination of Dairy Products."
3. Fundamentally, the English modified method as suggested by Wilson *et al.*

The data in hand suggest that the English method, which prescribes the careful inversion (not shaking) of each tube at 30-minute intervals, results in shortening the reduction times as well as in rendering them significantly more reliable as indicators. It is commonly recognized that the accuracy of the test as usually operated is impaired by the rising of the bacteria to the surface as the creaming process proceeds. The modified test yields more uniform bacterial dispersion brought about by frequent inversion.

The reduction times were much the same when methylene blue chloride and methylene blue thiocyanate were used in approximately equal concentrations. Significantly longer reduction times resulted when these dyes were used in concentrations of 1 : 300,000 than when used at concentrations of 1 : 700,000. J.M.F.

75. Bacterial Content of Milk as Affected by the Use of Different Plating Methods. ALEC BRADFIELD, Univ. of Vermont, Burlington, Vt. Vt. Agr. Exp. Sta. Bul. 417, April, 1937.

In this study tryptone-glucose-skimmilk agar was compared with standard agar at 32° C. (89.6° F.) and at 37° C. (98.6° F.) incubation temperatures using representative samples of both raw and pasteurized milks carry-

ing heterogeneous bacterial flora as well as milks contaminated from specific sources.

Greater increases in counts were attributed to lower incubation temperatures than to change in medium composition, although modified medium gave higher counts than did the standard.

The modified medium developed larger colonies and was more opaque which made counting easier and doubtless more accurate.

The modified medium plus 32° C. (89.6° F.) incubation gave higher counts in nine-tenths of the samples of raw and pasteurized milk, the latter giving three times as great an average percentage increase as the former.

Organisms derived from the udder, feces, hair, etc., apparently grew almost as well on the standard as on the modified medium. Those developed because of inadequate cooling or derived from unsterile utensils, feed and stable dust grew in significantly greater numbers at the lower incubation temperatures on both media but more particularly on the modified.

Skimmilk is an important ingredient in media, especially for plating pasteurized milk.

Dry milk solids, made by either the spray or flake process, appear to be as satisfactory for this purpose as does skimmilk, and may be added to the media in powder form together with the other ingredients.

On the whole, the Bowers-Hucker medium incubated at 32° C. (89.6° F.) proved to be more accurate and consistent than the standard as a means of estimating the number of bacteria in milk, and it is believed that milks of good quality will not be seriously penalized by its use as a basis of grading.

J.M.F.

76. Starters. W. Ritter and Th. Nussbaumer. Schweiz Milchzeitung No. 77, 1936.

After a short survey of the bacteriology of cream-acidifying cultures, the different methods for testing starters are discussed. Besides the test for bacterial pureness according to Burri's smear culture method, the following determinations are of first importance: the degree of acidity, the volatile acids, acetoin + diacetyl, eventually of carbonic acid and, above all, smell and flavor. Of the different methods of determining acetoin (acetylmethylcarbinol) + diacetyl, the most simple and most positive method is that of Hammer, but with an addition of α naphthol.

Starters may be cultivated for a long time without undergoing remarkable changes. However, an alteration may suddenly take place which is characterized by a strong increase of the amount of volatile acids and a simultaneous decrease of acetylmethylcarbinol and diacetyl. Attempts to bring on this change artificially were not successful.

Experiments on the methylene blue reduction time of starters in milk pasteurized in different ways gave shorter reduction-times for milk sub-

mitted to high pasteurization than for milk submitted to holding pasteurization. Likewise small amounts of copper (2 mg. per litre) produced a much more prolonged reduction-time in milk submitted to holding pasteurization than in milk submitted to high pasteurization.

In conclusion some experiences made with a starter consisting of only one organism (acidifying as well as acetoin-+diacetyl-forming) are made known. W.R.

BUTTER

77. Actual Methods Used in the Making of Danish Butter. J. M. Rosell, Dept. of Bacteriology of the Agr. Faculty, Montreal, Canada. Le Lait *17*, 161, p. 1, Jan., 1937.

Danish butter is characterized by its very desirable flavor. The flavor is obtained by use of high quality of cream in churning, by the use of butter culture and by proper care in all the culturing and churning operations. Neutralization of cream is not practiced although some washed cream may be used. Deodorization and removal of gases from cream is practiced in many creameries. The acidity at which the cream is churned is variable and is determined by the fat content of the cream to be churned. Details of the various steps in the manufacturing of Danish butter are reviewed. A.H.J.

78. A Holding Test at Room Temperature as an Indication of the Keeping Quality of Butter. D. H. Jacobsen. So. Dak. Bul. 308, 1937.

Interest is manifested in some means of determining or predicting the keeping quality of butter. Trials were carried out in which small samples were held at room temperature and the resulting flavor changes compared with those occurring at lower temperatures. Results obtained have indicated a need for further study.

This work included 40 churnings in which culture was used (15 salted and 25 unsalted) and 50 non-culture churnings (16 salted and 34 unsalted).

Particular attention was given to kind and extent of flavor deterioration and to kinds and numbers of bacteria which were present and which developed on holding.

Samples were held at 0°, 5°, 15°, and 21° C. The cream used in making the butter graded from sweet cream to 0.60 per cent acidity and samples were taken between September and June.

Results showed that a "holding test" consisting of holding small portions of unsalted butter for 7 to 10 days at room temperature (21° C.) gives useful information on the keeping quality of such butter at lower temperatures. The salted butter did not show flavor deterioration except for tallowiness within 7 days at 21° C., therefore no information was obtained on

the value of a holding test for the prediction of keeping quality of salted butter. The increases in numbers of total bacteria and the flavor deterioration in unsalted non-culture butter were much more extensive than in the unsalted culture butter held under similar temperature conditions. Salt in butter at the rate of 2.5 per cent effectively prevented the growth of lipolytic and proteolytic bacteria under the conditions of this investigation.

C.C.T.

79. Fishy Flavor of Butter and the Means of Checking It. W. RITTER. Schweiz. Milchzeitung No. 95–98, 1936.

A short report is made on the forthcoming of fishy flavor in butter and the means of preventing it. In Switzerland as well as elsewhere fishy flavor in butter could be kept down by omitting to acidify the pasteurized cream. It must, however, become possible to prevent fishy flavor also in butter made of acidified cream. For this reason, and in addition to Swedish and a great number of Swiss experiences, in the central plant at Lucerne were carried on during a certain period experiments on the influence of pasteurization-temperature on the forthcoming of fishy flavor in butter. From these experiments it followed that, if the cream was heated at 78, 80 and 82° C. in the flash pasteurizer, fishy flavor developed regularly after some time in the butter, although it was not salted. If the pasteurization-temperature was brought to 86° C., fishy flavor appeared in the butter after some time of storing in about half the cases. If the cream was heated at 90° C., fishy flavor was not found in the butter even after a storage of 10 weeks. The experiments were carried out with two maturators of 3000 litres of cream each. In order to keep down the cooking flavor of the cream, the difference between the temperature of the heating-water and that of the cream must be as slight as possible. This may be attained by increasing the number of plates, especially in the heat recuperating compartment. From different analogous cases it follows that the favorable action of an increased pasteurization temperature is due to the development of antioxidizing substances in the cream, not to a more radical destruction of the germs.

The results obtained at Lucerne respecting the influence of pasteurization temperature on the development of fishy flavor in butter are in agreement with other experiences concerning the keeping quality of Swiss butter made of acidified cream. W.R.

80. Investigations on the Phenomena Occurring During the Cooking of Butter (Ghee-manufacturing). W. RITTER AND T. NUSSBAUMER. Schweiz. Milchzeitung 7 to 11, 1937.

It was found that the butterfat obtained by evaporation of the water at high temperature contains lecithin. On the other hand, the butterfat ob-

tained by liberation of the buttermilk at a low temperature is free from lecithin. In the first case an intense foaming takes place a second time towards the end of the cooking process. In this moment the lecithin passes from the cooking residium into the foam in the form of brown flakes and is dissolved in the hot fat. During the cooling it is again precipitated as a brown, more or less flaky or slimy sediment. Fat which is free from lecithin becomes white when overheated. The presence of lecithin seems to be important also for the constancy against oxidation and it may equally delay to a certain extent the crystallization of the fat.

Butterfat is able to dissolve water. Fat that has been saturated with water at increased temperature becomes turbid when cooled, owing to the liberation of the water in the form of finest drops. The solubility of the water in the fat increasing with the temperature, it is difficult to remove the last traces of water without heating under reduced pressure or to long heating under ordinary pressure. Therefore such a butterfat contains generally still about 0.1 per cent of water. This water content may be somewhat reduced by filtration, etc.; it seems to be important for the reaction against oxidation and for the corrosion of the cans made of tinned iron. Especially in the latter case the way in which the liberated water is distributed becomes very important. The crystallization of the fat is different according to the kind of cooling. The fat ought to be as fine-grained as possible, as it is obtained by inoculating fat warmed at 30 to 33° C. with solid fat and keeping the mixture for some time at the above mentioned temperature. Further details on this subject will be given later on. W.R.

Other abstracts of interest are numbers 73, 76, 83, 84, and 95.

CHEESE

81. Research on a Secondary Odiferous Fermentation or Gray Decay of Gruyere and Emmental Cheese. W. Dorner and Marg. Thoeni, Federal Dairy and Bacteriological Inst. at Liebefeld-Berne. Le Lait *17*, 166, p. 561, June, 1937.

Odiferous fermentation or gray decay of cheese is a defect which appears 4 to 5 months or longer after the manufacture of the cheese. It is first evident as the pH of the cheese rises. This is eventually accomplished by a change in the odor and taste. At the end of a few weeks the odor becomes putrid and a grayish color develops. The cause of the fermentation was found to be *Bacterium proteolyticum* n. sp. When the milk used in cheese making contained as many of these organisms as 10 per cubic centimeter, secondary odiferous fermentation ensued. The properties of cheese that has been badly infected with the organism are a high pH value, a bad odor, a gray color or dark spots, and the liberation of gas. A.H.J.

82. The Industrial Control of the Fat Content of Cheeses. JEAN PIEN, Dir. of the Lab. of Farmer's Union, Paris, France. Le Lait *17*, 166, p. 569, June, 1937.

Methods of determining the fat content of cheese are (1) dissolving the cheese in an acid or alkaline solution and extracting the solution with ether; (2) grinding the cheese with sand or an inert mineral salt and extracting with ether; and (3) dissolving the cheese in a stannous chloride solution and then determining the fat by the Gerber-Roeder method. The first method was considered impractical for use in the industrial laboratory. The second method was considered satisfactory and superior to the third method as far as accuracy of results was concerned. The Gerber-Roeder method was considered the most rapid as a control method but the results have the possibility of being inaccurate to the extent of $\pm$ 1 per cent. A.H.J.

Other abstracts of interest are numbers 76 and 95.

CHEMISTRY

83. The Determination of the Diacetyl in Butter. JEAN PIEN, JACQUES BAISSE, AND ROBERT MARTIN. Dairy Lab. of Farmers Union, Paris, France. Le Lait *17*, 167, p. 675, August, 1937.

Of 130 samples of butter investigated 11 per cent contained diacetyl between 1.0 and 0.5 milligrams per kilogram. All the butters which were high in their diacetyl content were considered very desirable in flavor and aroma. However, many samples which were very low in diacetyl were also excellent in flavor and aroma. This would indicate that other butter constituents besides diacetyl are responsible for desirable flavor and aroma in butter. Since no butters were found in which the diacetyl naturally present exceeded 1.0 milligrams per kilogram, it may be judged that diacetyl has been added to butters containing larger quantities of diacetyl than this. The addition of diacetyl to the extent of 20 milligrams per kilogram would not be practiced as such addition affects deleteriously the flavor and keeping quality of the butter. The addition of 10 milligrams per kilogram gives a flavor that is somewhat too strong but this quantity of diacetyl might find use in the butter industry. The use of 1 to 5 milligrams per kilogram improved the flavor and aroma of butter and caused no deleterious effects in butter stored for 8 days at 20–25° C. (68–77° F.). Such butter had a more desirable flavor and aroma than the control butter containing no added diacetyl.

In determining whether diacetyl has been added to butter, the quantities sought should, therefore, be between 1 and 10 milligrams per kilogram. Various methods for determining this quantity of diacetyl were investigated. The method adopted consists in the reaction between diaminobenzidine and diacetyl which yields diphenylquinoxaline. This compound is yellow in

solution and the diacetyl can accordingly be determined by a comparison of the yellow color with standards made with potassium dichromate. The diacetyl is removed from the butter in a special distillation apparatus which allows its concentration in 10 cubic centimeters of liquid. To the 10 cubic centimeters of distillate from the butter is added 0.5 cubic centimeter of a 2.5 per cent aqueous solution of diaminobenzidine (freshly prepared). After agitation 0.5 cubic centimeter of concentrated hydrochloric acid is added. After shaking again, color comparison can be made in about two minutes. In order that the color be stable, it is necessary to use the reagents in the proportions given. In working with 100 grams of butter, diacetyl may be determined in amounts as low as 0.5 milligram per kilogram. A.H.J.

84. Detection and Determination of Diacetyl According to Pien, Baisse and Martin. W. Ritter and Ths. Nussbaumer. Schweiz. Milchzeitung No. 103, 1936.

The method of diacetyl determination according to Pien, Baisse and Martin is based on quinoxalic condensation of diacetyl (as Diketon) with diamins, in the present case m-p. toluylendiamin. Thereby a yellow dimethylquinoxalin is formed that can be determined colorimetrically. The method gives pure yellow colorings if pure reagents are used as ever fresh solutions. On the other hand, the method presents a disadvantage, that is to say: acetylmethylcarbinol (acetoin) which is present in the cultures must be oxidized first and has to be distilled in the form of diacetyl, just in the same way as in the nickel-diemthyl-gloxin-method. This is not necessary in the Voges-Proskauer-method carried out according to Hammer by means of Kreatin and solution of sodium hydroxide, to which some α-naphthol may be added. W.R.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

85. The Influence of Irradiation on the Content of Vitamin A in Concentrated Milks. G. Genin, Paris, France. Le Lait *17*, 164, p. 368, April, 1937.

Irradiated evaporated milk was found to have the same vitamin A content as the same evaporated milk not irradiated—*i.e.*, 4.28 units of vitamin A per gram of milk. Ultra-violet irradiation of the milk accordingly did not reduce the vitamin A content of the milk. A.H.J.

86. Physico-Chemical Modifications of the Constituents of Milk in the Course of Concentrating in the Presence of Sugar Syrup. Michael Polonovski. Le Lait *17*, 167, p. 699, July–August, 1937.

Sweetened condensed milk was found to yield excellent results in infant feeding. An explanation was sought in the physico-chemical changes oc-

curring during the manufacture of the milk. Sweetened condensed milk had a higher viscosity and a higher surface tension and a decreased flocculability than sweetened pasteurized milk. The buffer value underwent only a small variation and the change was inconsistent. It is difficult to associate any of these changes with the better utilization of milk in infant nutrition. The decreased flocculability with associated softer and less rapidly forming curd is probably the most significant factor. The changes in surface tension and viscosity are probably associated with more profound changes in the protein molecule and their relationship to infant nutrition has not been studied. A.H.J.

87. An Automatic Procedure for the Making of Milk Powder. G. GENIN, Paris, France. Le Lait *17*, 165, p. 488, May, 1937.

The Gray Jensen process for the manufacture of milk powder is described. A.H.J.

88. A New American Plant for the Manufacture of Lactic Acid. G. GENIN, Paris, France. Le Lait *17*, 163, p. 259, March, 1937.

The manufacture of lactic acid and sodium and calcium lactates from whey is described. The milk sugar in the whey is fermented to lactic acid by a mixed culture of *Lactobacillus Bulgaricus* and a mycoderm. When the acidity of the fermenting whey reaches 1.3 per cent, lime is added to neutralize the acid and the fermentation allowed to continue. It is usually necessary to add lime 4 times during the fermentation of a tank of whey. About 48 hours are required to convert the lactose in the whey to lactate. At the end of the fermentation, an excess of lime is added and the mass heated to 90° C. (194° F.) in order to precipitate the lactalbumin. The precipitate is removed by passing the liquid through a filter press. The filtered liquid is clarified by the use of activated carbon and then concentrated and cooled whereupon the calcium lactate crystallizes. The calcium lactate may be further purified by dissolving, re-decolorizing, concentrating, and re-crystallizing. In order to prepare lactic acid the calcium lactate solution is treated with sulphuric acid whereupon calcium sulphate precipitates. The lactic acid is obtained by filtering off the calcium sulphate. By modifying the procedure lactic acid of 22 and 44 per cent concentration may be obtained. Sodium lactate is prepared by neutralizing the lactic acid with caustic soda. A.H.J.

89. The World Production of Casein. G. GENIN, Paris, France. Le Lait *17*, 168, p. 605, June, 1937.

World production of casein was 60,000 tons in 1932 and 70,000 tons in 1936. The chief uses to which the casein is put are as plastic material, glue,

paper coating, in insecticides, in the textile industry, in casein paints and as a food material. In the United States two-thirds of the casein produced is used in the paper industry. The United States, France and Argentina are the chief countries producing casein, these countries each producing about 18,000 tons annually. A.H.J.

Other abstracts of interest are numbers 73, 74, 75, 90, 91, 92, 93, 95, 104, 108, 110, 115, 118, and 119.

DISEASES

90. Mastitis—Tests for Detecting Its Presence in Milk. K. G. WECKEL, Univ. of Wisconsin. Milk Dealer *26*, 12, p. 70, Sept., 1937.

The author relates the effect of mastitis on milk and describes some of the tests for determining the presence of mastitis in the herd. C.J.B.

91. Mastitis. V. The Presence of Mastitis Streptococci in Bovine Mammary Tissue. G. J. HUCKER, N. Y. Agr. Exp. Sta., Geneva, N. Y. Tech. Bul. 241, Jan., 1937.

"In order to determine the prevalence of mastitis streptococci in bovine mammary tissue, 46 udders were aseptically removed from cows and virgin heifers and cultured. Only udders were studied in which no evidence of infection could be noted by gross examination of the udder prior and subsequent to removal. Inasmuch as only mastitis-free cows from the standpoint of physical and post-mortem examination were used, a considerable number of animals were observed to secure the 46 used in the present investigations.

A study of 24 udders aseptically removed and cultured from cows known to be free of mastitis and to have passed through one or more lactation periods shows that all contained mastitis streptococci.

A similar study of 21 udders removed from virgin heifers and calves indicated that less than half contained mastitis streptococci in the mammary tissue." G.J.H.

92. Mastitis. VI. The Effect of Feeding Irradiated Yeast on the Resistance of the Udder to Bovine Mastitis. G. J. HUCKER AND MARION SNYDER REED, N. Y. Agr. Exp. Sta., Geneva, N. Y. Tech. Bul. 243, July, 1937.

"To determine the effect of irradiated yeast on the resistance of dairy cows to udder infections, 116 cows in four dairy herds were studied. Fifty-one were fed irradiated yeast in the grain mixture. In three herds 9 ounces per day per cow and in one herd 4½ ounces per day per cow were fed.

Prior to the beginning of the feeding of the yeast, weekly quarter samples from all cows were subject to laboratory examination over a 4-month

period to determine the amount of mastitis infection present. Examinations were made for presence of mastitis streptococci, number of leucocytes per cc, reaction to brom thymol blue, and physical appearance of the milk. Subsequent to the initiation of the yeast feeding, similar samples were secured for approximately 20 months. In all, the observations were made on the experimental herds over a period of 2 years.

In general the feeding of irradiated yeast was found to have no significant effect upon the resistance of the udder to the invasion of mastitis streptococci. Depending upon the index of infection used, from 10 to 13 per cent more of the infected yeast-fed cows showed an improvement than was found in the case of the infected cows not fed yeast.

No prophylactic effect was found by the addition of irradiated yeast to the diet of mastitis-free cows." G.J.H.

93. Mastitis. VIII. The Use of a Specially Prepared Vaccine in an Attempt to Control Bovine Mastitis. G. J. HUCKER AND P. A. HANSEN, N. Y. Agr. Exp. Sta., Geneva, N. Y. Tech. Bul. 245, July, 1937.

"A study was made of the possible prophylactic and therapeutic action of a vaccine prepared from stock and freshly isolated herd strains of *Streptococcus agalactiae* Lehmann and Neumann. Injections of milk were used in connection with the vaccines.

One hundred and two animals in four herds were studied over a period of 2 years, 45 being vaccinated and 57 being retained as untreated controls.

Weekly quarter samples were secured for 4 to 7 weeks prior to vaccination and weekly quarter samples for 3 months and monthly quarter samples for approximately 14 months subsequent to vaccination were examined in the laboratory for presence of mastitis streptococci, number of leucocytes per cc., reaction to brom thymol blue, and physical appearance of the milk.

It is concluded that vaccines prepared from stock and freshly isolated strains of *Streptococcus agalactiae* give no evidence of increasing the resistance of dairy cattle to mastitis.

Similar vaccines were found to have little or no therapeutic action in the treatment of latent and chronic udder infections. Neither the prophylactic nor therapeutic action of this vaccine was stimulated by the use of simultaneous intramuscular injections of milk." G.J.H.

FEEDS AND FEEDING

94. Animal Nutrition. LEONARD A. MAYNARD. McGraw-Hill Book Co., New York, 1937, pp. 483. Price $4.00.

This book, according to the preface, is based upon twenty years experience in teaching the principles of animal nutrition to graduate and under-

graduate students at Cornell University. It was written primarily to give a systematic knowledge of these principles and not to give specific directions for feeding farm animals. Such being the case the book must impart more knowledge about feeding rats than cows as so many of the facts of nutrition were gained in this way.

The various chapters of the book deal with the expanding field of nutrition, the animal body and food, physical bases of life processes, carbohydrates and their metabolism, lipids and their metabolism, proteins and their metabolism, inorganic elements and their metabolism, vitamins, determination of digestibility, nutrition balances, nutritive energy, maintenance, growth, reproduction, lactation, and work production. A considerable portion of the book deals with chemistry and physiology. For example, the chapter on carbohydrates devotes eight pages to the classification and chemistry of carbohydrates of special interest in animal nutrition, and the chapter on lactation is much concerned with the anatomy of the udder and the physiology of milk secretion. A knowledge of this scientific background is essential to a thorough understanding of the general subject of nutrition.

The author has succeeded particularly well in developing an interesting style. Innumerable facts on the problems confronting the field of nutrition, on special applications to human nutrition, on pioneer contributions of many individuals and the author's interpretations give special interest that even the college student may read certain passages without the oppressive remembrance that he is studying. This book is very well organized and the subject-matter has been carefully selected. A.C.D.

95. The Weston-Levine Vitamin Chart, Seventh Revision, Sept., 1937. Roe E. Remington, Medical College of the State of South Carolina, Charleston, S. C.

This chart, originally published in 1931, has been revised annually. By careful selection and condensation of authentic information it summarizes the latest available data as to the composition and properties, principal functions, results of deficiency, and food sources of the vitamins.

In the seventh revision note has been taken of the most recent progress in vitamin research, particularly in the identification of the various factors of the B complex, including Williams' synthesis of vitamin B_1. Flavin is substituted for vitamin G and the P P factor added. The results of vitamin deficiency have been classified as specific and non-specific, and the list of food sources checked with results appearing since the last edition. The list of references has been limited to those most likely to be of value to students of the subject.

The chart is offered for sale by the author. R.E.E.

96. Bacteriological Investigations About Ensiling Green Forage with the Addition of Acid Whey, Skimmilk or Sugar. J. VAN BEYNUM AND J. W. PETTE. Report of the Experimental Dairy Farm, Hoorn, Holland, pp. 1–38, 1937 (English summary).

This work is a continuation of studies reported in 1935 to improve grass silages by cultured whey, skimmilk, or buttermilk.

The pH is the principal controlling factor in silage quality. It must be 4.2 or less. If higher *clostridium tyrobutyricum* attacks the lactic acid converting two molecules into one of butyric acid, thereby increasing the pH and promoting spoilage.

The lactobacilli are more important than the lactic streptococci as the latter do not produce enough acid. Whey added to the grasses is beneficial by increasing the moisture content to facilitate the exclusion of air and by increasing the sugar content for lactic fermentation. A.C.D.

97. Experiments with a Practical Silage Problem Dealing with the Addition of Dairy Wastes or Sugar. E. BROUWER. Report of the Experimental Dairy Farm, Hoorn, Holland, pp. 39–102, 1937 (German summary).

It has been established that a knowledge of pH of silage is invaluable. For example, a pH value of 4.5 or above indicates poor quality, a value range of 4.0 to 4.5 places the silage in a doubtful class, while good fermentation feeds have a pH of 4.0 or lower. However, a silage with a pH value of less than 4.0 may be detrimental for cheese purposes. This is especially true when certain organisms and spores are present.

It has been learned that the sense of smelling is most valuable in classifying silage. Sweet odors are desirable and mild clean acid odors are most objectionable. It is even possible to classify a silage as good that has a fruity odor. A rotten or butyric acid odor must not be present. The odor of certain esters in silages places them in a doubtful class. Classifying silage on the basis of odor is regarded as being comparable or better than doing so with numerous analyses as a guide.

The experiment dealt with the addition of 3 to 10 per cent of whey to silage. Wooden, stone and iron silos were used. With this procedure less than 6 per cent of the 121 silos studies produced a silage with a pH value of less than 4.0 and 62 per cent of the samples had pH values greater than 4.5. Butyric acid and ammonia were present in many samples. Many samples had a very bad odor which was readily transmitted to the milk or its products.

The author points out that the identification of organisms found in silage is not important, but that a knowledge of the kinds that grow in the silage is the important item.

Comparable results were obtained with silos and pit silos. The argument that a tall sweet silo will guarantee good silage is a falacious assumption.

The ammonia content of grass silage increased greatly with rising pH values. At a pH of 4.0, 8 per cent of the total protein was ammonia; at a pH of 5.0, 24 per cent of the total protein was ammonia. This same relationship held for other silage feeds.

Silage with a pH of 4.0 contains very little butyric acid but with a rise in pH the butyric acid content increased. This held in a lesser degree with other silage feeds than with grasses.

Bean silage was not eaten freely by cattle. Various silages gave comparable results from the point of view of the health of the animals.

Nineteen trials were conducted with the addition of 0.5 per cent of sugar to the grass, 15 earth silos were used, 3 silos and one Feimen (stacked). Only 4 trials gave pH values of 4.0 or lower, 9 gave pH values higher than 4.5.

Whey or 0.5 per cent of sugar added to silages did not improve it according to 140 trials made in a practical way. J.C.M.

98. Bacteriological Examination of Silages Prepared with Addition of Whey, Skimmilk and Sugar on Farms in Netherland. J. Van Beynum and J. W. Pette. Report of the Experimental Dairy Farm, Hoorn, Holland, pp. 103–157, 1937 (English summary).

There are three types of grass silage made without the addition of inorganic acids. Type A silage has not heated in fermentation, hence a lactic acid fermentation lowered the pH below 4.2 because plenty of sugar was present, and the silage is of good quality. If the added sugar was not well distributed there will be a bad spotty butyric acid fermentation in the low sugar areas. In Type B silage the sugar content was too low and fermentation ceased above pH 4.2. Then *clostridium tyrobutyricum* fermented the lactic acid into butyric and the old silage became putrid. Type C silage heated during fermentation and destroyed both lactic and butyric bacteria. It is much like spontaneously heated hay.

Most grass silages with added whey are Type B for the sugar content is too low. Their pH gradually increases with age to 6 and the quality decreases. By added sugar the pH may be made sufficiently low to keep well. The acid distribution is better in sugar silage than in A. I. V. silage. Type C silage should not occur when whey is used.

In 1934 grass silages with one per cent added sugar, with and without cultures, did not keep well. Best results have been secured recently by adding both whey and sugar or an increased amount of sugar.

A.C.D.

99. Artificial Hay-making. G. Genin, Paris, France. Le Lait *16*, 160, p. 1061, Dec., 1936.

Young grass is dried in a heated chamber to yield a hay that is high in protein, vitamin A, ash constituents, and other food elements necessary for

good growth of young animals and for high milk production in dairy cows. Four cuttings of young grass per year materially increase the yield of hay. Because of the intensive cropping, the use of fertilizer is recommended. The feeding of artificially dried young grass makes it unnecessary to use more expensive concentrates in the ration. The cost of an artificial drying may be reduced appreciably by exposing the young grass to sunshine and then finishing the drying in the artificial drier. This allows some loss in feed value but dried young grass is still of excellent quality. A.H.J.

100. Virgin Rice Bran in the Feeding of Dairy Cows . . . Experiments of 1936. Tolesforo Bonadonna, School of Zootechnology of the Faculty of Vet. Med., Milan, Italy. Le Lait *17*, 170, p. 1025, Dec., 1937.

Virgin rice bran when pure and properly cared for, *i.e.*, devoid of all foreign matter capable of adulterating it, is an excellent and economical feed for the nutrition of dairy cattle. In the proportion of 40 to 80 per cent of the ration (from 1 kilogram to 1.5 kilogram and more per day) rice bran exerted no harmful effect on the health of the cows even during hot weather. From the point of view of cheese making, "Reggio" type, rice bran, provided it was pure and not fermented, did not have any harmful effect. The maturing of the cheese was, however, more rapid when rice bran was included in the ration of the cow. Rice bran also influenced certain properties of the butter, but the change depends on the nature and composition of the other feed given with the rice bran. A.H.J.

ICE CREAM

101. The Bacteriological Quality of the Ice Cream Supply for a Small City. M. W. Yale and R. C. Hickey, N. Y. Agr. Exp. Station, Geneva, N. Y. Tech. Bul. No. 248, Sept., 1937.

With the exception of three or four of the larger cities in New York State, but little work is being done by municipalities on ice cream sanitation, and knowledge in respect to sanitary quality of ice cream and ice cream ingredients is quite inadequate.

A bacteriological study was made of the ice cream supply for a small city since it was believed that the results would be of general interest and applicable to the situation in many other municipalities.

Both total and coliform counts were determined for 77 process samples from 7 local plants, 137 ice cream samples from 12 retail stores, and 36 dipper water samples from 18 establishments. Process samples demonstrated that either gelatin or color was excessively contaminated at four plants and cream at one plant. Freezing equipment was in poor sanitary condition at two plants.

Coliform counts were more sensitive than either standard nutrient agar or tryptone agar counts in revealing contamination by ice cream dippers and dipper waters which were in poor sanitary condition in the majority of instances.

Three of the 12 manufacturers had all standard agar plate counts of store samples of vanilla ice cream under 100,000 per gram. Eight manufacturers had an average (logarithmic) standard plate count under 100,000 per gram. The average count of 112 store samples was 59,800 per gram, a higher average than usually found in cities and states where bacteriological control is exercised. In the case of 9 of the 12 manufacturers, there had been no previous bacteriological control of their product. M.W.Y.

Other abstracts of interest are numbers 73, 74, 75, 78, 86, 95, 103, 113, and 119.

MILK

102. The Use of a Tincture of Guaiacum in the Control of Pasteurization. B. Leibovitch, Chief of the Lab. of the St. Hubert Dairy at Nancy, France. Le Lait *17*, 165, p. 463, May, 1937.

Various chemical methods of determining the effectiveness of pasteurization were investigated.

A preparation consisting of 100 cubic centimeters of acetone, 1 gram of gum guaiacum, and with or without from ½ to 3 grams of guaiacol was considered to yield satisfactory results. The use of guaiacol made the reagent more sensitive. With ½ gram of guaiacol in the reagent, milks flash pasteurized at 76 and 77° C. (168.8 and 170.0° F.) showed positive reactions while when pasteurized at 78, 79, and 80 (172.4, 174.2 and 176.0° F.), the reaction was negative. However, when 3 grams of guaiacol are used in the reagent only the milks flash pasteurized at 79 and 80° C. (174.2 and 176.0° F.) show negative results. These and other data indicate that peroxidase in the milk is not destroyed even on flash pasteurizing at 79° C. (174.2° F.). On using ½ gram of guaiacol in the reagent, the milks which gave negative reactions when flash pasteurized at 78, 79, and 80, (172.4, 174.2, and 176.0° F.) gave positive reactions when 1 per cent of raw milk was added. Where flash pasteurization is practiced, it is considered that this text supplies a control measure that will allow the production of a truly hygienic milk.

A.H.J.

103. The Bacteriological Standard for Pasteurized Milk. A. L. Provan. Milk Industry *17*, 6, p. 45, Dec., 1936.

The growth of many bacteria found in pasteurized milk can be greatly increased by the addition of one per cent milk to the agar media.

Memo. 139/Foods (Jan. 1937) issued by the Ministry of Health gives details of the test to be used for control purposes. The new order states that pasteurized milk shall contain not more than 100,000 bacteria per ml. as determined by the colony count on a medium which contains one per cent of milk. The new media now recommended by the Ministry of Health (1936 order) is:

Yeastral	3 gr.	Whole milk	10 gr.
Peptone	5 gr.	Water	1,000 gr.
Agar	15 gr.		

Dr. Provan in anticipating this order in 1935 used a media which was slightly different from that now recommended by the Ministry of Health in order to determine the effect that the addition of milk might have on the results obtained on pasteurized milk.

The media he used contained 3.0 gr. of Lemco in place of the yeastral and 10.0 gr. of skimmilk in place of whole milk now recommended. In comparing his media with the Standard Agar (1923 order) he found that the milk agar often gave greater counts than did the plain agar. The higher the count on the plain agar the greater the percentage of samples showing a decidedly higher count on the milk agar.

There is some evidence that heat resistant organisms present in the raw milk will be of greater importance when milk agar is used for determining the count. L.H.B.

104. The Numbers and Kinds of Bacteria in Aseptically Drawn Milk. H. R. Thornton and N. J. Strynadka. Twenty-Fourth Ann. Report of the Intern. Assoc. Dairy and Milk Inspection, p. 178, Oct., 1935.

Milk was aseptically drawn into sterile flasks from 95 cows in 12 herds. Two were from fresh cows (within 48 hours after parturition) and were labeled colostrum milks. Five were from cows having mastitis and were labeled mastitis milks. Eighty-eight were milks normal in appearance. One thousand fields were examined per smear. Of the 88 normal samples of milk 31 contained less than 500,000 leukocytes per cc. and 57 contained more than 500,000 per cc.

The average Breed count for the 31 samples containing 500,000 or less leukocytes per cc. was 34,980 and the average plate count was 918 and the average Breed count for the 57 samples containing over 500,000 leukocytes per cc. was 204,515 and the average plate count was 36,625. No rod shaped bacteria were observed in any of the 95 samples of milk examined.

Only single cocci were found in 35.5 per cent of the low leukocyte count milks and in 15.8 per cent of the higher leukocyte count milks.

Groups no larger than diplococci were found in 38.7 per cent of the low leukocyte count milks and in 24.6 per cent of the high leukocyte count milks.

Streptococci (three or more in chain) were found in 16.6 per cent of the low leukocyte count milk and in 42.1 per cent of the high leukocyte count milk.

Clumps (three or more in non-chain formation) were found in 22.6 per cent of the low leukocyte count milks and in 26.3 per cent of the high leukocyte count milks. L.H.B.

105. The Milk Supply of the Nation. Hygienic Production and Control. BEN DAVIES, Dir. of the Lab. for United Dairies, London, England. Le Lait *17*, 165, p. 449, May; 166, p. 591, 1937.

The importance of proper hygienic control of milk production is emphasized. The determination of the bacterial counts of pasteurized milk rather than raw milk is suggested as a control measure as in milks properly produced in equipment that is not satisfactorily cleaned, the bacterial count may even increase on pasteurization. The pasteurization of milk of every quality is recommended. A.H.J.

106. The Utilization of Tubercular Milks. J. VERGE AND G. THIEULIN, Vet. School at Alfort, France. Le Lait, *17*, 164, p. 348, April, 1937.

According to French law milks containing tuberculosis organisms or originating from animals afflicted with tuberculosis cannot be used for food of man or animals either as such or as manufactured products unless they are heated sufficiently to destroy the tuberculosis organisms.

A.H.J.

107. Colon Bacilli and Bacteriophages of Milk to be Consumed in the Raw State in Warsaw. IRENE LIPSKA, Municipal Inst. of Hygiene at Warsaw, Poland. Le Lait *17*, 163, p. 236, March, 1937.

The colon bacilli of winter milk as well as of summer milk are such as are characterized by biochemical properties intermediate between the species *Bacterium coli commune* and *Bacterium lactis aerogenes*. The filtrates of summer milk were more active with respect to colon bacilli of milk and with respect to animal and human excrement and urine of those afflicted with kidney maladies than those of winter milk. There was observed an analogy in the activity of the milk for colon bacilli and for typhoid and paratyphoid bacilli. The greater extent of the activity of the filtrate from summer milk included 16 strains from 23 bacilli tests. The most frequently attacked were the colon bacilli from animal and human excrement; those of milk were more resistant, the most refractory being the pathogenic colon bacilli isolated from urine. A.H.J.

108. The Production of a Clean and Healthful Milk on the Farm. A. TAPERNOUX, Prof. of Chemistry at the Vet. School at Lyons, France. Le Lait *17*, 163, p. 241, March, 1937.

The chemical and biochemical composition of milk is discussed. The production of a clean and healthful milk on the farm means the elimination of infected animals from the herd, the exercise of proper milking technique, and the maintenance of satisfactory sanitary conditions in the barn. A.H.J.

109. Actinization of Milk. JEAN VIEILLY AND JEAN HARDER, Doctors of Veterinary Medicine of the Industrial Dairy at Grenoble. Le Lait *17*, 166, p. 576, June; 167, p. 707, July–August, 1937.

Actinization is defined as a treatment with ultra-violet rays for the hygienic purpose of destroying dangerous organisms and the conservation of lactic acid organisms and for the nutritive purpose of increasing the vitamin D potency. The actinizator of Stoutz is described in which the milk is irradiated as it flows through quartz tubes. In the actinization of milk, it becomes sterile at the exposed surface proportional to the time exposed to the rays, bacterial colonies are destroyed, and chemical and physical modifications are imparted to the milk. Infants appear to tolerate actinized milk better than normal milk, the digestibility appears to be improved, and the vitamin D potency is increased. A.H.J.

110. The Preservation of Dairy Products by the Procedure of Hofius. G. GENIN, Paris, France. Le Lait, *17*, 167, p. 727, July–August, 1937.

Unpasteurized milk at about 8° C. (46.4° F.) is placed in a steel cylinder so that it is three quarters full. Oxygen under 8 atmosphere pressure is then introduced into the cylinder and the milk shaken. The valve on the cyclinder is then opened and the oxygen pressure relieved. The outgoing oxygen carries with it any gases dissolved in the milk. Oxygen is again introduced into the cylinder until a pressure of 8 atmospheres is attached. The cylinder containing the milk is then placed at a temperature of 8° C. (46.4° F.) and shaken for 2 hours. If the pressure in the cylinder is still above 7 atmospheres the cylinder of milk is placed in storage at 3 to 6° C. (37.4 to 42.8° F.), otherwise the oxygen pressure is raised to 8 atmospheres before the milk is stored. The milk will keep and there will be no increase in acidity during a storage period of 14 days. If the milk is heated rapidly but not pasteurized before being subjected to the oxygen treatment it will keep for 4 weeks. When such milk is held for 42 days there will be a considerable development of off-flavor in the milk. If the storage temperature of milk treated by the Hofius process attains 18 to 22° C. (64.4 to 71.6° F.), the acidity will show an increase at the end of 3 days. Milk serum keeps less well by the Hofius process than whole milk. The off-flavor which develops in whole milk treated by the Hofius process is that of caramelization; in milk serum, a malt-like flavor. Peroxides have not been detected in milks treated by this process. A.H.J.

111. The Purification of Milk in the Dairy Industry. The Necessity for Filtration or the Clarifying Centrifuge. Advantages and Disadvantages. J. GIROUX, Director of the Lab. for the Control of Milk and Chemical and Bacteriological Research of the Maggi Dairy Society. Le Lait *17*, 164, p. 354, April, 1937.

The removal of foreign matter from milk by filtration or by the clarifying centrifuge is necessary to assure complete freedom from sediment. The centrifuge is more satisfactory than the filtration but filtration is a simpler and less expensive operation. Neither of the two methods of purifying milk remove significantly large amounts of milk constituents from the milk. The clarifying centrifuge remove about 80.5 milligrams per liter of milk of which the composition is 33.9 per cent dry matter, 2.09 per cent fatty matter, 22.23 per cent total nitrogen (as casein), 4.08 per cent carbohydrate, and 5.5 per cent ash. When calculated to the fluid milk these losses do not constitute a very large percentage. During the centrifuging the slime which is formed carries with it most of the impurities that have gotten into the milk. Neither the action of the centrifuge nor of filtration removes the bacterial flora from the milk. Centrifugation has the advantages of security, constant milk flow, possibility of working at low temperature, more effective removal of debris and animal cells and economy of operation. Filtration has the advantages of being simple, non-mechanical, and excludes air more effectively from the milk. Filtration has the disadvantages of requiring heated milk, of not removing suspended material so well, of requiring frequent replacing of the filtering tissue, and difficulty of cleaning. Before pasteurization or sterilization, the practice of both filtration and clarifying centrifugation of the milk or at least one of them is desirable. A.H.J.

112. Consideration on Bulgarian Curdled Milk Prepared from Sheep's Milk. I. KVATCHKOFF. Le Lait *17*, 165, p. 472, May, 1937.

Various organisms that are used in making cultured milks are discussed. Methods of manufacturing cultured milk are given as is also the chemical composition of the finished products. Properly made Bulgarian curdled milk had excellent keeping quality. Such milk of the proper acidity will keep for a month or more when stored at low temperature. This makes it possible to ship the product made from sheep's milk to countries where sheep's milk is not available. Bulgaria has an annual production of about 2.5 million hectoliters of sheep's milk. A.H.J.

113. The Influence of Metals on Milk. W. RITTER. Schweiz. Milchzeitung No. 59–60, 1936.

After discussing prevailing experiences, the author reports about his own experiments. In order to make the influence of metals more evident, the

milk was submitted to holding pasteurization, cooled, and stores for 24 and 48 hours. Metals are more apt to produce tallowy flavor in milk when in contact with it during the holding pasteurization and not only during storage thereafter. Copper and all copper-alloys (brass, bronze, German silver, monel metal) develop tallowy flavor when the milk is treated in this way. Nickel leaves a vitiated, not distinctly tallowy flavor in milk. Aluminum, tinned and chromed material, and stainless steel do not affect the flavor of milk.

When determining the aldehyde-reductase (xanthine oxidase) in milk submitted to holding pasteurization in the presence of metals, the strongly damaging influence of copper and all its alloys becomes again evident. This influence is even exerted, though in a very slight degree, by tinned copper and tinned German silver. All these copper-containing metals are distinguished by a more or less prolonged reduction time, according to their yielding of copper.

The copper contents of a metal can also be detected by applying high pasteurization to milk treated with metals and submitting the milk to the peroxydase-reaction after cooling it. This procedure reveals again the presence of all copper-containing metals in milk by a rapid setting in of a positive reaction with dimethyl-p-phenylendiamin-sulphate, α-naphthol and hydrogen-peroxide.

In a similar way, but more feebly, copper in all its alloys influences the nadi-reaction of the heated milk (dimethyl-p-phenylendiamin-sulphate and α naphthol, without hydrogen-peroxide).

The non copper-containing alloys, even if they are able, as for instance nickel, to produce a vitiated flavor in milk, do not affect the aldehyde-reductase (xanthine oxidase), peroxydase- and nadi-reaction. W.R.

114. The Influence of Bacterial Activity on the Peroxydase-Reaction of Copper-Containing Milk. W. RITTER. Schweiz. Milchzeitung *89*, 1936.

Small amounts of copper in milk can be traced by heating the latter rapidly to 85° C. and submitting it afterwards to peroxydase-reaction with dimethyl-p-phenylendiamine-sulphate, α-naphthol and hydrogen-peroxide. An important bacterial content of the milk is liable to influence this reaction in two different ways; Slackening it by reducing the easily reducible indophenol dye which is formed, or intensifying it by accelerating the nadi-reaction through different, bacteria. The bacteria being for the most part killed by high pasteurization and a renewed infection being scarcely probable, this case presents a merely theoretical interest.

On the other hand an intense bacterial activity taking place before pasteurization is liable to exert a slackening influence on the peroxydase-reaction also after pasteurization, if there are only very small amounts of

copper. As soon as the copper content is but slightly increased, the copper reveals itself all the same, although somewhat slowly. Acid milk cannot be used; even milk which is not acid, but too ripe, is apt to prevent the setting in of the reaction. This phenomenon is probably due to the effect of certain chemical substances able to resist high temperature. It follows that only fresh milk or cream should be submitted to the peroxydase-reaction for the detection of copper. W.R.

115. Control of Onion Flavors in Milk. C. E. WYLIE, Univ. of Tenn. Milk Dealer *27*, 1, p. 84, Oct., 1937.

The author briefly discusses the means of controlling onion flavor by (1) removing the onion flavor from milk, (2) getting the cow to throw off the onion flavor before she has produced the milk, (3) preventing the cow from getting onions, and (4) eradicating the onions from the pasture. C.J.B.

116. New Phosphatase Test Checks Efficiency of Milk Pasteurization. L. H. BURGWALD, Ohio Agr. Exp. Sta., Wooster, Ohio. Ohio Agr. Exp. Sta. Weekly Press Bul. No. 22, 36, Nov. 11, 1937.

The phosphatase test of Kay and Graham is depicted as a boon to the public health officials and control laboratories of commercial dairies. It is pointed out that the test can determine discrepancies of 1.0° F. in heating temperatures and variations as little as 5 minutes in holding time. Also, as little as 0.1 per cent of raw milk added to properly pasteurized milk can be detected by this test. W.E.K.

117. Farmers Can Gain By Understanding Milk Market Plans. Dept. of Rural Economics, Ohio Agr. Exp. Sta., Wooster, Ohio. Ohio Agr. Exp. Sta. Weekly Press Bul. No. 22, 40, Dec. 9, 1937.

Studies in Akron and Stark County markets show that many farmers market their excess over base milk through channels outside the market pool. A warning is issued urging farmers to understand fully the base allotment selling plan in order to avoid reduction in their base allotments. W.E.K.

118. Some Unusual Flavors in Milk. W. D. DOTTERRER, Bowman Dairy Co., Chicago, Ill. Milk Dealer *27*, 1, p. 54, Oct., 1937.

The author briefly discusses some of the unusual flavors in milk, such as chloro phenol, oxidized, and those caused by bacterial action. C.J.B.

119. Methods of Measuring the Bacteriological Efficiency of Milk Plants. E. H. PARFITT, Purdue Agr. Exp. Sta., Lafayette, Ind. Milk Dealer *27*, 1, p. 58, Oct., 1937.

The author suggests several methods for measuring the bacteriological efficiency of a milk plant. These methods include those which determine

the ability of the plant operations to obtain as effective destruction of the organisms contained in the milk as secured under laboratory conditions, those methods which determine the extent of increase of count due to contaminated equipment, and those methods which can be used to determine thermophilic contamination. C.J.B.

120. Summary of Present Legal Opinions of Milk Control Legislation. SAMUEL B. WEINSTEIN, Oregon Milk Control Board. Milk Dealer *26*, 12, p. 43, Sept., 1937.

The author reviews court decisions regarding milk-control legislation from 1904 to the present time. C.J.B.

121. Certified Milk as a Source of Vitamin C. W. H. RIDDELL AND C. H. WHITNAH, Kansas State College, Manhattan, Kansas. Cert. Milk *11*, 126, p. 9, Oct., 1936.

These investigators point out that fresh milk is a more important source of vitamin C than it is generally assumed to be. The effects of ration, freshness, method of pasteurization and exposure to sunlight on the vitamin C content of milk are given. W.S.M.

Other abstracts of interest are numbers 73, 74, 75, 76, 83, 84, 85, 86, 90, 91, 92, 93, and 95.

PHYSIOLOGY

122. Lactation and Glycemia. JULIO LORENZO Y DEAL, Director of the Hospital Pereyna Rossell, Montevideo, Uruguay. Le Lait *17*, 162, p. 113, Feb., 1937.

The glycemia of the nursing mother varies so that the blood may show from 0.130 to 0.051 per cent sugar. No relationship appears to exist between the sugar content of the blood and the abundance of milk secretion, although higher milk yields coincide with blood sugar contents of 0.087 and 0.067 per cent. There appears to be no relation between glycemia and the age of the nursing infant. In the different phases of milking, following the glycemia at the beginning, middle, and the end of the milking it was found that in the cases of glycemia below normal, the diminution during milking was very slight, for glycemia above the normal, the milking had considerable influence on glucose-lactose metabolism and reduced the sugar content of the blood, and in cases where the sugar content of the blood was normal, both increases and decreases in blood sugar occurred during milking. A.H.J.

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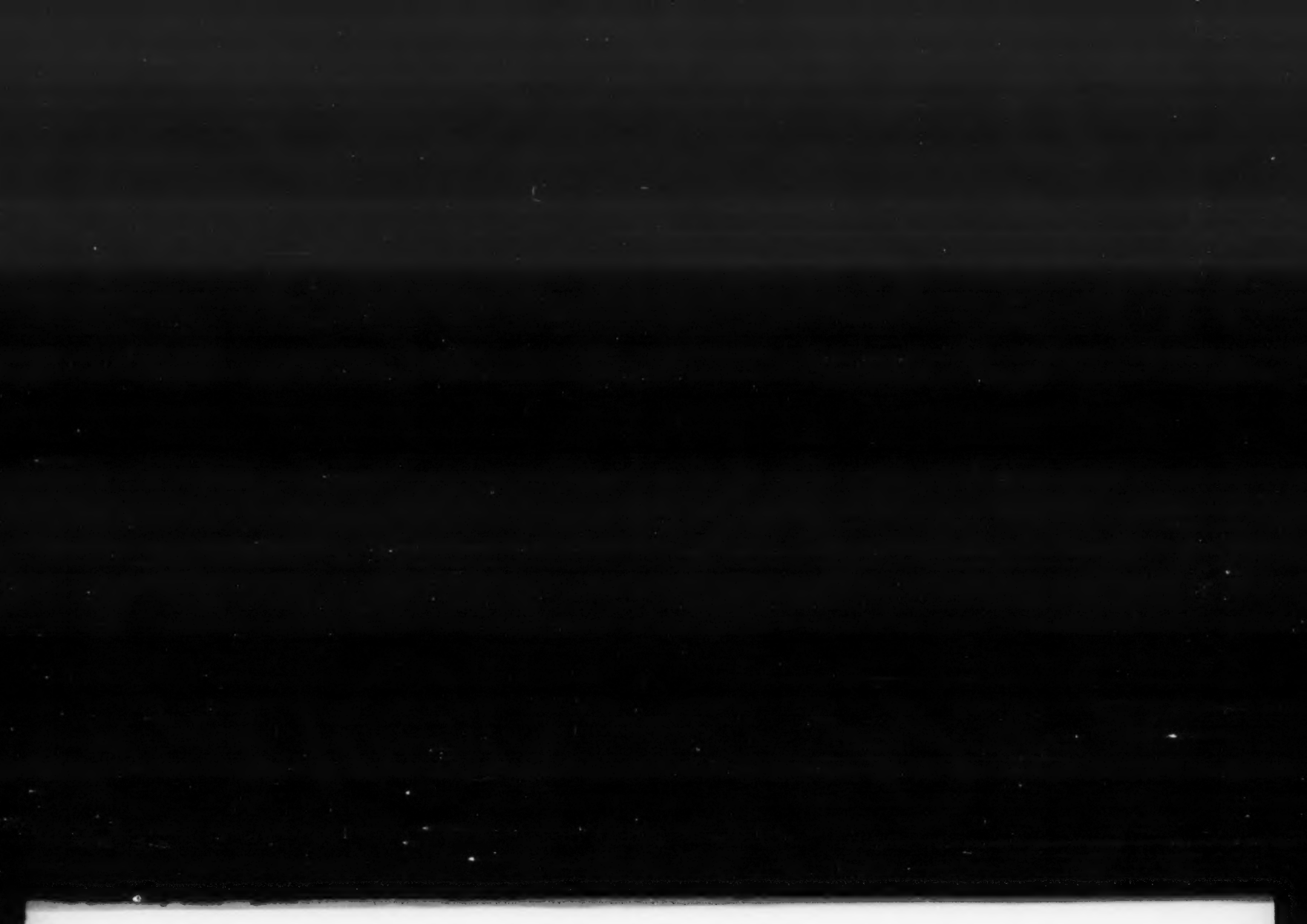

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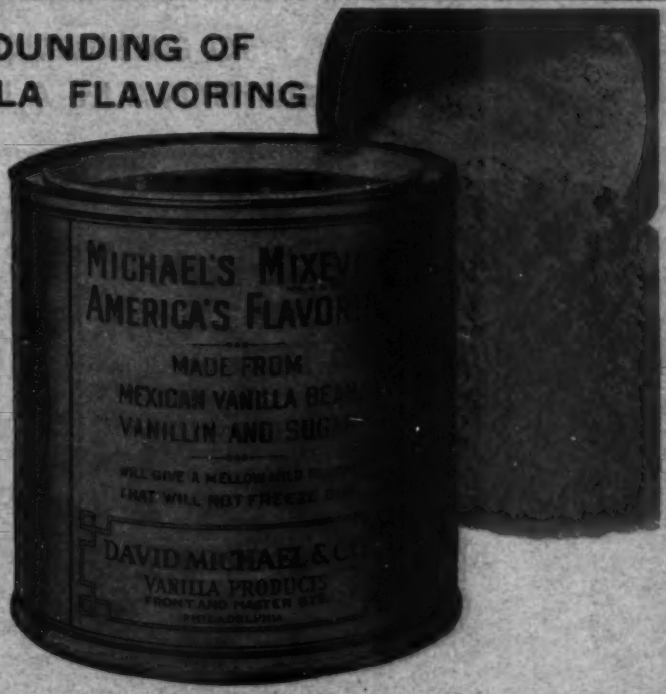

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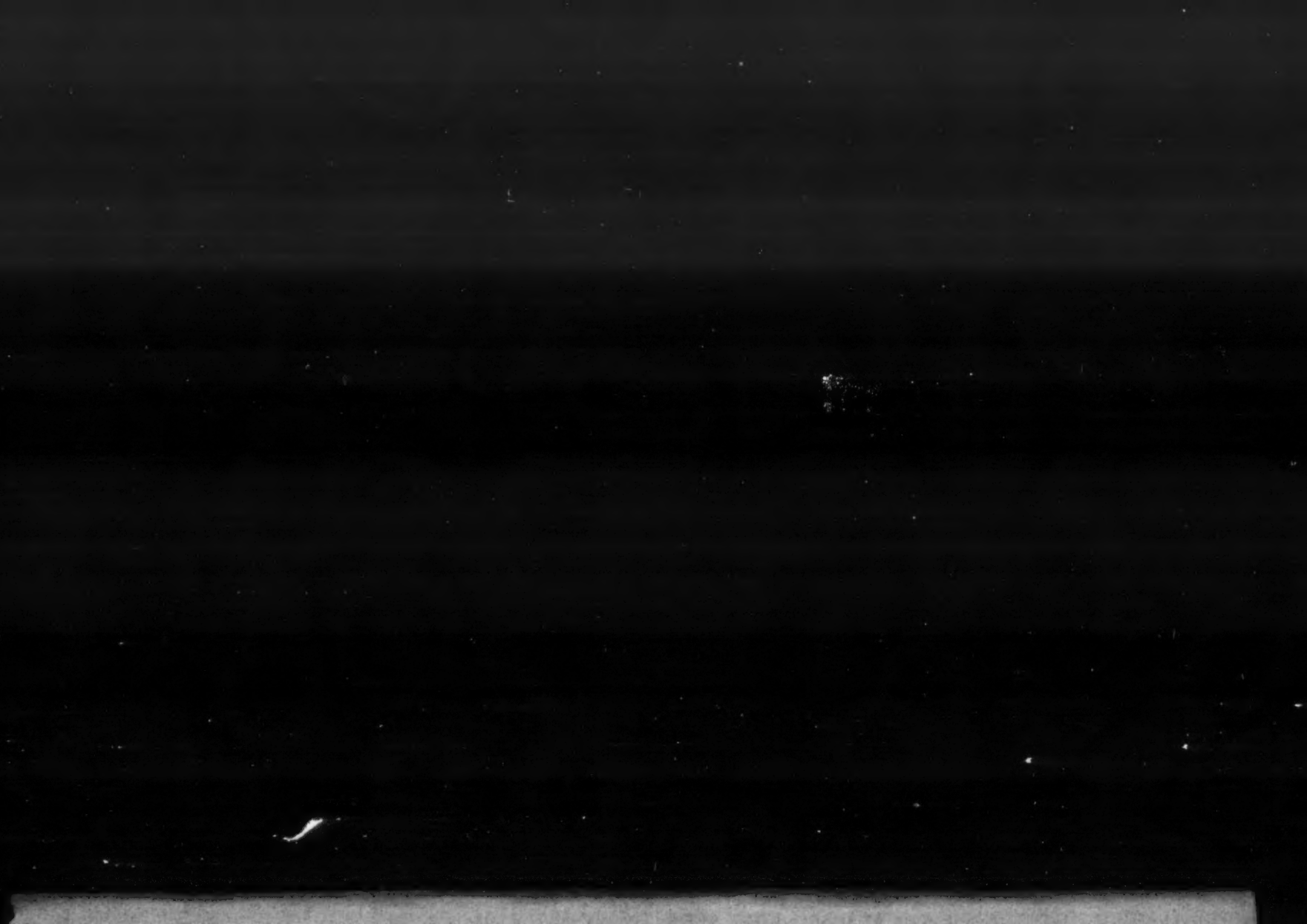
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